INTRODUCTION

I. VERY BRIEF RETROSPECTIVE

Drugs, especially poisons, have been around as long as animals have sought relief from physical and mental pain. Primitive humans both observed animals in their consumption of native materials and experimented themselves. Ronald Siegel in his book Intoxication (E.P. Dutton, 1989) goes so far as to postulate that the desire to achieve an altered state of consciousness is a drive we share with the lower animals. Originally steeped in magic, the human use of drugs is as old as recorded history. Egyptian medical records written on papyrus around 1500 B.C. (the Ebers Papyrus) included over 800 recipes for the therapeutic and toxic uses of such natural materials as opium, digitalis(from foxglove),heavy metals (such as lead and silver, not music), and atropine. Some of these prescriptions were effective, many were not. The ancient Romans, Greeks and Chinese used many types of drugs, herbs and drug formulations as well as poisons. Who does not know the fate of Socrates? Or if you saw the PBS series "I, Claudius" you would be aware of the deadly activities of the Roman empress, Livia, the wife of Caesar Augustus.

The Greek physician Galen (120-100 A.D.) made fashionable prescriptions which were a combination of ingredients (does this sound familiar?). He also concocted extracts called "galenicals". His mixtures represent some of the first recorded incidences of polypharmacy. The theories and experimentation of Galen influenced therapeutics for the next 1500 years.

antidote

One of the classic combinations was an **antidote** (an agent which directly counteracts the action of a drug or poison) taken by King Mithridates VI of Pontus. This ruler was so afraid of being poisoned (an alternative to despotic rule) that he ingested the mixture as a **prophylactic** (preventative) measure. The potion worked so well that when he tried to kill himself by poisoning the attempts failed. He eventually had to use a less sophisticated means of suicide. To this day an archaic term synonymous with is **mithridate**.

prophylactic

mithridate

The Middle Ages (400-1500 A.D.) were a time of Arabic and Jewish contributions to the pharmacopeia. The alchemical advances of the Middle East were passed on during the Arabic invasions of Europe of that time. It should be kept in mind that the Far East also had its systems of medicine and pharmacy of which acupuncture and herbalism still remain and are still under investigation in terms of their efficacy and underlying scientific truths.

The early Renaissance period heralded the beginnings of formal therapeutics in the work of Paracelsus (a pseudonym) (1493-1541). A reprobate by reputation, the Swiss physician is considered to be the

Grandfather of Pharmacology. He formalized the concept of a **toxicon** as a distinct chemical entity whose properties, therapeutic and toxic, were dose dependent and could be determined by scientific experimentation. At the same time, the sophistication of poisoning was enhanced in the infamous activities of the Borgias.

toxicon

The advancement of medicine and chemistry complemented and were complemented by pharmacology during the eighteenth and nineteenth centuries. The modern age of pharmacology might be marked by the research of Paul Ehrlich (1854-1915), the Father of Chemotherapy. Winner of the 1908 Nobel Prize in Medicine and Physiology for his pioneering work in immunology, Ehrlich developed the concept of drugs which were tissue and cell specific - his magic bullets. Although no magic bullets existed in Ehrlich's day, the concept guides drug research to this day.

Picture of Paracelsus fro the National Museum of Medicine.

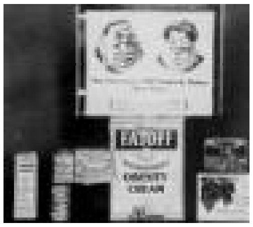
And what of the primitive peoples of the earth, those of the Amazon jungle and the wilds of New Guinea? There exists a vast wealth of untapped information of which only the shamans, curanderos and medicine men and women are aware. Native American Nations are credited with using intravenous injections long before its formalized introduction around the time of the Civil War. How much of this wealth of knowledge is being destroyed by the deforestation the Amazon jungle?

Evidence of the importance of as yet unanticipated information on naturally occurring agents, the U.S. Department of Agriculture in late 1991 granted Bristol-Myers Squibb Company the authority to harvest the bark of Pacific yew trees in federal forests for the chemical taxol, a potential anticancer drug. Soon after other natural and synthetic precursors were developed so that the natural sources would not be depleted.

Table I documents a brief chronology of important therapeutic advances since the end of the 18th century. We should keep in mind that there could exist a parallel listing for the development of toxins used in warfare and agriculture.

II. DRUG REGULATION

Pre-twentieth century society had few restrictions as to the types, purity or amounts of substances which could be dispensed for the ills of its citizens. There were isolated laws devised by individual municipalities to protect their populations from food contaminants and extremely toxic materials. But legislation on a national scale was instigated mainly during this century. During the late nineteenth century herbal remedies, Dr. Hall's snakeoil, mercury, arsenic, strychnine could all be bought "over-the-counter" from anyone who went to the trouble to formulate and package them. "Caveat emptor" was a reality. The Industrial Revolution plunged the major world cultures into technological change and public awareness which necessitated some kind of code of law relating to foods and drugs.



NMM

Table 1

A Brief Chronology of Drug Investigation and Development

1796	Jenner performs the first successful human vaccination against smallpox.
1804	Morphine is first isolated by Armand Sequin.
1817	Emetine is identified as the active principle in ipecac.
1820	Quinine is isolated.
1842	Diethyl ether is publicly used as a general anesthetic.
1864	Barbituric acid is synthesized by Adolph van Baeyer.
1903	The first barbiturate is introduced - barbital.
1922	Banting and Best perform the first successful injections of purified insulin to control diabetes mellitus in humans.
1928	The antibacterial activity of penicillin is discovered by Alexander Fleming.
1930s	The medical use of amphetamines begins.
1935	Gerhard Domagk publishes the chemical information of the first sulfa drug, Prontosil®.
1952	Chlorpromazine (Thorazine®) ushers in the widespread use of tranquilizers for victims of mental disease.
1960s	The age of oral contraceptives begins.
1980s	The products of recombinant genetics are marketed - insulin, growth hormone, tissue plasminogen activator.
1990s	Retrovirus therapies are developed; vaccines for hepatitis, chicken pox and mumps become common; gene therapy is born.
Late 1990s	Breakthroughs in the causes and treatments for Alzheimer's Disease, diabetes, the spongiform encephalopathies, and breast cancer are made.

NMM

A. Drug Legislation

Listed here are some key pieces of United States legislation which relate to the status of drug, food, and other substance development today.

1. **Federal Pure Food and Drug Act** (1906)

This was a ground-breaking piece of legislation which was concerned mainly with drug purity and sanitation in the processing of foods.

2. **Federal Food, Drug and Cosmetics Act** (1938)

The law of 1906 was only a beginning. It did not regulate the safety and effectiveness of drug formulations. This was no more evident than in the case of mass poisoning which resulted from the consumption of "elixir of sulfanilamide" produced by the S.E. Massengill Company of Bristol, Tennessee. Between September and October 1937 doctors had prescribed almost 12 gallons of a 10% solution of sulfanilamide in an ethylene glycol(antifreeze) solvent flavored with saccharin, caramel, amaranth, and raspberry extract. The company's chief chemist, Harold Watkins, was not aware of the toxicity of that concentration of the solvent. He was merely trying to present the drug, used effectively to combat gonorrhea, meningitis and strep throat, in a more palatable form, especially for children. One hundred seven persons died, most of them children. This tragedy helped to move a stalled piece of legislation through the Congress which required drug and food producers to prove that all their products were safe. As the title implies it also specifies that cosmetics should be harmless. It established a governmental regulatory body, the Food and Drug Administration (FDA) and set The National Formulary and The United States Pharmacopeia as "official compendia" of drug information. Amended many times, this law stands today as the basis for our current drug development procedures.

The foods section of the legislation sets standards for food for both humans and animals. It has three subdivisions which pertain to illegal foods, legal additions to foods(additives), and the labeling of food items. The portion governing drugs has parts pertaining to new drugs, prescription drugs, over-the-counter drugs, biological drugs, and medical devices. The third section concerning cosmetics specifies that they must be safe, labeled truthfully as to the ingredients and manufacturer's name and address, and that they must be packaged under sanitary conditions.

A footnote to this history - under the 1906 law the Massengill Company and Watkins could only be charged with improper labeling. Watkins was subsequently fired and in early 1939 committed suicide.

Significant Amendments

✔ Durham-Humphrey Act(1952) - This amendment defined specific drugs which may be supplied by a licensed pharmacist upon the prescription by a registered physician. Under this act these drug prescriptions may not be refilled unless authorized by the physician.

✔ Food Additives Act (1958) - This act included the infamous "Delaney Clause" which in effect says that substances used as food additives must be classified as human carcinogens if they lead to cancer when ingested by man or animals at any level of exposure. The additives used prior to 1958 were placed on a list and called Generally

Recognized As Safe (GRAS) because of their previous unchallenged use.

According to a June 3, 1991 report in Chemical and Engineering News, an estimated 5.5 billion dollars worth of additives are currently used in the U.S. market alone. When a new additive is developed it must show "demonstrable safety in animal studies" before it can be placed on the GRAS list. Fewer than ten new chemicals have received approval by the FDA in the last twenty years.

✔Kefauver-Harris or Drug Amendments Act (1962) - In 1953 a small German chemical company synthesized and studied a compound which seemed to have potential in the fledgling field of sedative-hypnotic drugs. Although the drug was tested for toxicity it took several years and many thousands of prescriptions in Europe to discover that the sedative and antinausea drug, called thalidomide, caused gross malformations in growing fetuses. It should be noted that a 1939 addendum to the original 1938 legislation made testing for toxicity and teratogenicity mandatory for all new drugs. This prevented thalidomide from being marketed in the United States. As a result of the thalidomide tragedy the Kefauver-Harris Act added more provisions for extensive pre-clinical drug evaluations such as reports of chemistry, pharmacology, toxicology, formulation data, quality assurance, and manufacturing procedures as well as more thorough clinical investigations. Before submitting a New Drug Application (NDA) the potential drug developer has to file an Investigational New Drug application (IND) which outlines the plans for the testing and development of the proposed drug.

This law also mandated that the producers of all types of drugs, whether prescription or over-the-counter, would have to prove their efficacy and safety. Note that over 3000 prescription drugs and drug combinations had been developed between 1938 and 1961. Countless others went into over-the-counter(OTC) preparations. However, an investigation was launched to study all existing prescription drugs. The final report in 1971 found that 15% of all such drugs were ineffective, 35% were possibly effective, 7% were probably effective, 19% were effective and 24% were effective with limitations. These findings are still highly debated. **OTC**

In 1972 the Federal Drug Administration (FDA) initiated an OTC drug study. The final report of seventeen advisory panels was issued in 1983. Only one-third of the 700 active ingredients in some 300,000 OTC products were found to be effective. Then in November 1990 the FDA banned 223 drugs found in OTCs because their therapeutic claims were unsubstantiated. All of the products affected had been in use since before 1962.

✔ Antidrug Abuse Act (1988) - This and similar state laws established criminal penalites for the distribution or possession of anabolic-androgen steroids except for the treatment of disease. Violators are subject to the penalities imposed by the Comprehensive Drug Abuse Prevention and Control Act of 1970 (see below).

3. **Hazardous Substances Labeling Act** - 1967

4. **Comprehensive Drug Abuse Prevention Control Act** - 1970 - This act established a hierarchy or schedule of prescription and prohibited drugs.

Schedule I - These materials possess high abuse potential and currently have no acceptable use in the United States. These may not be prescribed. Permission for experimentation with these substances must be obtained by the Drug Enforcement Administration. Examples of Schedule I drugs are heroin, marihuana, peyote, mescaline, psilocybin, LSD, Ecstasy.

scheduled drug

Schedule II - The drugs at this level also have a high abuse potential and could cause psychic or physical dependence. They may be prescribed but are under stringent control. Schedule II drugs include opioids(morphine), amphetamines and methamphetamines used alone or in combination as well as some barbiturates.

The following Schedules can be prescribed by physicians and dentists.

Schedule III - These chemicals have less abuse potential that those in the first two categories but their abuse may lead to moderate or low physical dependence or high psychological dependence. Examples are certain opiods in limited quantities (codeine), and some nonopiods such as glutethimide(sedative), phendimetrazine(diet aide), and some barbiturates.

Schedule IV - This class represents medications considered to be of low abuse potential with the possibility of limited dependence especially when compared to the previous schedules. Examples are phenobarbital, chloral hydrate, the benzodiazepine tranquilizers, propoxyphene and meprobamate (all are sedatives).

Schedule V - The abuse potential of this class of drugs is the lowest of all. They may be dispensed by a pharmacist without a prescription providing certain conditions exist. The types of medications indicated are preparations with moderate amounts of opiods used for their antitussive or antidiarrheal properties.

The label of drugs in Schedules II through IV must read

> Caution: Federal law prohibits the transfer of this drug to any person other than the patient for whom it was prescribed.

Physicians and pharmacists prescribing and dispensing Scheduled drugs must register annually with the Drug Enforcement Agency (DEA), keep an accurate inventory of Scheduled drugs, and comply with a list of other stipulations.

5. **Occupational Safety and Health Act** (OSHA) (1970)

6. **Poison Prevention Packaging Act** (1970)

7. **Federal Controlled Substances Act** (1971) - Limits the availability of drug precursor substances.

8. **Consumer Product Safety Act** (1972)

9. **Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)** (1972) - Under this law pesticides already on the market are currently undergoing extensive scrutiny with regard to their testing especially with respect to carcinogenicity. Also being debated is the cost of disposal of existing agricultural chemicals which have been banned.

10. **Toxic Substances Control Act** (1976) - This umbrella legislation covers the handling and deposition of chemicals within the entire United States.

11. **Orphan Drug Act(**1983; amended 1984) - There are about 5000 diseases and conditions each of which affect relatively small populations(<200,000) of our citizens; for example, Huntington's disease, multiple sclerosis, narcolepsy, amyotrophic lateral sclerosis (Lou Gehrig's disease), leprosy, blepharospasm, adrenoleucodystrophy, AIDS, and inherited metabolic disorders such as PKU. Because of the expense of drug development and small potential market there was little financial incentive for pharmaceutical companies to develop drugs for the treatment of these conditions. This act provided incentives to develop and market orphan drugs by entitling a producer to

orphan drug

- 7 years of marketing rights after FDA approval;
- tax credits for clinical research expenses;
- assistance through the approval process by an FDA Orphan Products staff;

- grant support for clinical trials;
- waivers of the ≥ $250,000 filing fee for prescription drugs.

It also encouraged joint industry-university research efforts.

Only 10 products were approved for orphan conditions before the ODA; since 1983 there have been 108 products approved to treat more than 8 million patients, most of whom are children.

(See "The Impact of the Orphan Drug Act" **Modern Drug Discovery**, Sept/Oct 1998 and/or go to the web at http://www.fda.gov/orphan and http://www.rarediseaases.org .)

12. **Drug Price Competition and Patent Term Restoration Act** (1984)- Because of the extensive testing and red tape involved in developing new drugs, very few materials isolated or synthesized on the bench top actually make it to the market place. A single drug may takes an average of twelve years from its birth to final marketing with and average cost of about $300,000,000. The average time for FDA approval from the end of testing averaged 31.2 months. See Table 2.

Table 2

Number of New FDA-Approved Drugs Per Year

1959-61	52
1969-71	13
1972-74	16
1980	12 (out of 5700 offered for investigation)
1981	27
Jan 1998 -Aug 99	38

In December 1984 new regulations were issued which cut the time for approval, made it easier for companies to gain approval for generics whose patents had expired, lengthened the time allowed for patents in proportion to the amount of time spent in the FDA approval process, decreased the time a drug would have Investigation New Drug or IND status, proposed an Abbreviated New Drug Application (ANDA) for proposed generic drugs, and allowed studies performed in other countries to be submitted in evidence. **IND**

The current route for drug development is outlined in Figure 1 and can be found on the web at http://www.FDA.gov/cder/handbook . In 1988-89 regulations were loosened with respect to drugs proposed for the treatment of AIDS-related illnesses so that new drugs could be prescribed before all of the extensive testing was finished. This is referred to as the "fast" or "parallel track".

This is a very small but significant sample of the laws which govern the substances which we and our animals experience in our everyday lives. Issues which have yet to be satisifactorily addressed include timely notification of the availability of drugs, especially those on the fast tract, as well as disemmination of adverse side effects and dose requirements. The advent of biotechnological products has also made a significant impact on the legislative process.

Congress passed The Food and Drug Administration Modernization Act of 1997 in which an appeal was made to the public at large to update and streamline the FDA's public health mission (see http://www.fda.gov.oc/fdama/comm/).

Abuses and tragedies still occur. In 1985 The FDA Center of Veterinary Medicine launched an investigation into the widespread illegal use of drugs by veterinarians and farmers. It has been estimated that >20,000 drugs are currently being used in agriculture while only about 2500 have been approved by the FDA. Many of these drugs are from foreign sources, some are counterfeit, other have been diverted from destruction when they become expired in potency. In 1989 several employees of the FDA were prosecuted for taking bribes and several companies were castigated for falsifying information.

And what of the 5,000,000 known chemicals used as solvents and precursors in industry? Of these, 53,500 are regulated or inventoried by the Environmental Protection Agency (EPA) or FDA. Not all or even most have been assayed for toxicity or carcinogenicity. The National Toxicology Program (NTP), an agency within the Department of Health, Education and Welfare has been given the task of carrying out an organized study of the industrial chemicals currently in use. As you might imagine this is a truly awesome task. (http://ntp-server.niehs.nih.gov/)

Most other developed countries of the world have laws and procedures which direct drug development. Some are not as stringent as those of the United States. This is why you may have seen newspaper accounts of celebrities like Rock Hudson traveling to France for AIDS treatment. There is a current tendency for some medications to be more easily adopted in the United States if they have a history of use in other countries as, for example, with RU-486 (of course, controversy didn’t help its case).

- The world community has its own Scheduled chemical list with regard to agents of chemical warfare. Schedule I refers to those substances used exclusively for warfare such as the nerve gases, sulfur mustards, nitrogen mustards, and Lewisites. These are overtly banned.
- Schedule II includes direct precursor chemicals which must be monitored and recorded.

- Schedule III contains commercial chemicals which must also be monitored and recorded but which remain controversial because of their wide use in all types of industry.

It should be noted that some of the nitrogen mustards are also used for cancer chemotherapy and that a significant contribution to insecticide use was offered by the predecessors to today's nerve gases.

B. Federal Agencies

(Taken from Ottoboni, M.A. The Dose Makes the Poison 2nd Edition, Van Nostrand Reinhold Pulishers 1991)

The following is the alphabet soup of governmental agencies which monitor and regulate foods, drugs, cosmetics, agricultural chemicals, industrial chemicals, working conditions, and most of anything else with which we come in contact.

1. **Food and Drug Administration (FDA)** - an agency of the Department of Health and Human Services. It is responsible for the regulation of food additives, all processed foods (human and pet), drugs, biologicals, cosmetics, medical devices, advertising of prescription drugs. (http://www.fda.gov/) **FDA**

2. **U.S. Drug Enforcement Administration (DEA)** - under the Department of Justice. Its mission is to enforce narcotics and controlled substances laws and regulations as well as enforces those regulations. **DEA**

3. **Environmental Protection Agency (EPA)** - in the Department of Labor. It is in charge of protecting the environment through the regulation of all pesticides, pesticide residues on raw agricultural products, drinking water standards, air, water and soil pollutants, toxic water materials and all synthetic chemicals. The EPA is an independent agency that administers the Solid Waste and Emergency Response Programs such as the Superfund toxic waste cleanup and the Community Right-to-Know programs. (http://www.epa.gov/) **EPA**

4. **Department of Transportation (DOT)** - an entity to itself. It is responsible for the regulation of all hazardous chemicals as defined by DOT regulations while they are in transport. (http://www.dot.gov/) **DOT**

5. **Occupational Safety and Health Administration (OSHA)** - also in the Department of Labor. Its mission is to develop and enforce **OSHA**

regulations for workplace standards, conduct inspections, protect workers from chemicals and blood-borne diseases. (http://www.osha.gov/)

6. **Department of Agriculture (USDA)** - again its own agency. It is commissioned to regulate all commercial meat and meat products, poultry and dairy products. Veterinary services officials administer the regulation of veterinary biologics. A subagency within the USDA has a new group - Biotechnology, Biologics and Environmental Protection - which coordinates biotechnology matters. (http://www.usda.gov/) **USDA**

7. **Consumer Product and Safety Commission (CPSC)** - under the Department of Labor. Its goal is to protect the consumer against unreasonable risks from products specified by legislation such as the Hazardous Substances Labeling Act, Consumer Product Safety Act, and Flammable Fabrics Act. (http://www.cpsc.gov/)

8. **Federal Trade Commission (FTC)** - supervises the regulation of advertising of nonprescription drugs. (http://www.ftc.gov)

In addition to these groups, there are some other agencies which affect health care products such as the Centers for Disease Control (CDC), National Institutes of Health, National Institute of Standards and Technology, Patent and Trademark Office, U.S. Custom Service, and Bureau of Alcohol, Tobacco and Firearms. The American Conference of Governmental Industrial Hygienists (ACGIH) is a nonprofit professional association whch provides scientific information on the effects of occupational exposure to chemical and physical agents. The information provided is not intended for use as legal standards, but is generally accepted by labor, industry and regulatory agencies.

In April 1999 the CDC announced the ten most signficant public health achievements of the 20th century. They were

- Vaccination
- Motor-vehicle safety
- Safer workplaces
- Control of infectious diseases
- Decline in deaths from coronary heart disease and stroke
- Safer and healthier foods
- Healthier mothers and babies
- Family planning
- Fluoridation of drinking water
- Recognition of tobacco use as a health hazard

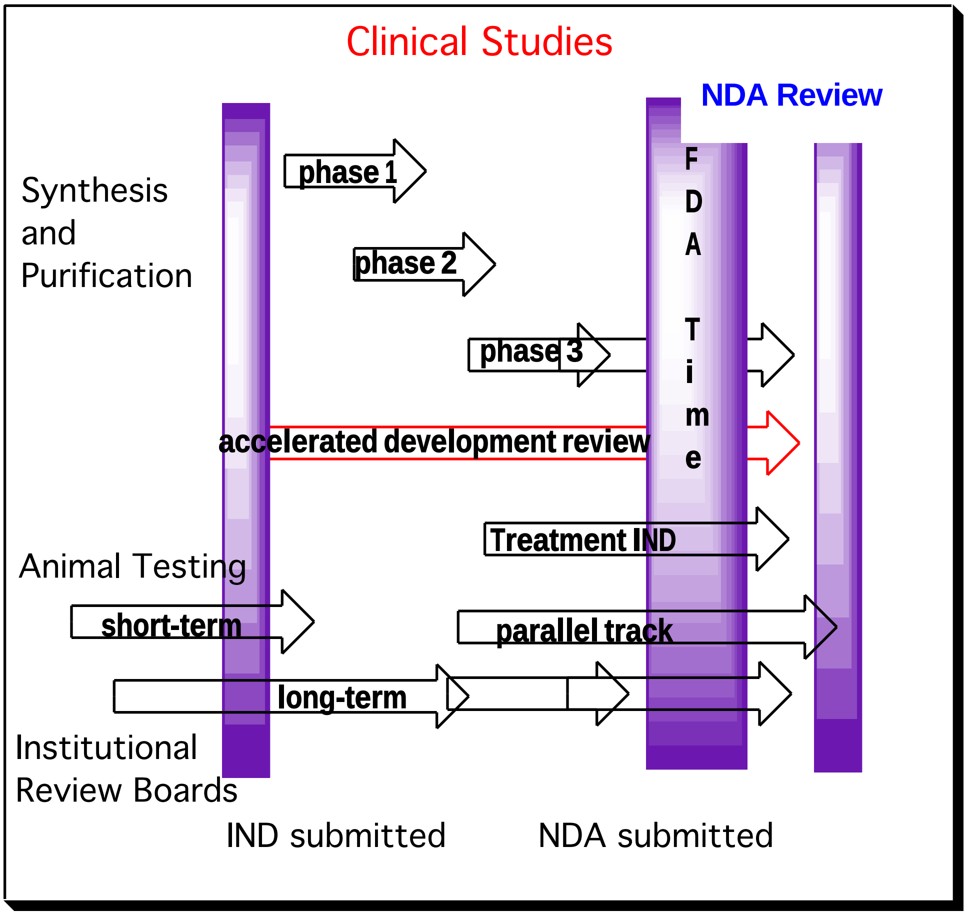
III. DRUG DEVELOPMENT

A. Timelines

The path which must be followed in the development of a material from lab bench to consumer is long, torturous and expensive. As stated before it takes about twelve years and over $200 million to bring an entity to the point of being marketed. Part of that timeline is understandable considering the laws governing safety and efficacy. Outlined in Figure 1 are the major steps on the way to a pharmaceutical product.

B. Procedures

To give you some idea of the intricacies of only a "small" (relatively) early portion of the process, consider the following protocols for toxicity testing.



modified from FDA 1998
http://www.FDA.gov/cder/handbook/

Figure 1: The Drug Development Process

1. Testing for Toxicity

a. Underlying Principles

* Effects produced in laboratory animals, when properly qualified, can be applied to man.
* Exposure of experimental animals to high doses to toxic agents is a necessary and valid method discovering possible hazards in man.
  e.g. To determine a 0.01% hazard (20,000 in 200 million) would require 30,000 animals. However, if the dose is increased several thousand times, fewer animals would be needed.

b. Descriptive Animal Toxicity Tests

* Acute - single exposure over a very short period of time
  - Oral LD_{50} (gavage) - pilot range study first; chemical administration to fasting animal in varying doses, observation , and autopsy after death.
  - Acute dermal toxicity-LD_{50}-uses rabbits
  - Acute inhalational toxicity-LC_{50}-protocol similar to oral
  - Primary eye irritation- rabbits as test animals - new in vitro cell testing
  - Primary skin irritation- uses rabbits
  - Skin sensitzation-uses guinea pigs-involve a large number of possible techniques, which indicates the inadequacy of any one.
* Subacute
  - the toxicity is observed at the highest dose; there should be no toxicity evident at the lowest dose.
  - Protocol - two weeks of dosing, observation, and analysis.
* Subchronic
  - Protocol - thirteen weeks, three different doses, controls, two species (rats and dogs of both sexes), and adherence to intended route of exposure.
  - Observations: mortality, diet, weight, urinalysis, hematology, clinical chemistry panel, gross and microscopic appearances.
* Chronic
  - Duration of tesing depends upon intended exposure in man; it can take as long as six months or may extend to the lifetime of the animal. Enough animals are needed to ensure 50% survival after an extended period of time. The rest of the protocol is similar to that of the subchronic study.
* Fertility and Reproductive (Phase I)
  - Two or three subtoxic doses given to female and male rats at prescribed times before mating to coincide with sperm and ovum production.
  - Observations will include percent pregnancy, number of stillborn and live offspring as well as weight, growth and survival during the first three weeks of life.
* Teratogenic (Phase II)
  - Protocol is similar to that of fertility testing using rats and rabbits.
  - Additional exposure is given at various stages of fetal development. Fetuses are removed by cesarian before normal parturition.

- Observations include the number of implantations, the number of living and dead fetuses, weight, measurement, gross and histological measurements.

* <u>Perinatal and Postnatal (Phase III)</u>
  - Gestation, delivery and lactation periods are observed. Protocol is otherwise similar to fertility studies.

* Multigenerational Reproduction Study
  - Usually uses three generations of rats with three dose levels and controls.

* Mutagenic
  - Bone marrow analysis
  - Dominant lethal
  - Ames Test

* Other Tests
  - Pharmaco- Toxico- kinetics
  - Antidotes

2. Mutagenicity and Carcinogenicity Testing

For the mutagenicity analysis a decision-point approach to testing for carcinogenicity is used(From Casarett and Doull, <u>Toxicology</u> 3rd Edition)
This approach begins with short-term testing followed by evaluation and further step-wise tesing with subsequent cumulative evaluations.

Stage A	Structure-activity relationships
Stage B	Short-term ***in vitro*** tests 1. mammalian cell DNA repair 2. bacterial mutagenesis (Ames test) 3. mammalian mutagenesis 4. chromosome tests (sister chromatid exchange) 5. cell transformation
DECISION POINT ONE	
Stage C	Test for promoters 1. ***in vitro*** 2. ***in vivo***
DECISION POINT TWO	
Stage D	Limited ***in vivo*** bioassays 1. altered foci induction in rodent liver 2. skin neoplasm induction in mice 3. pulmonary neoplasm induction in mice 4. breast cancer induction in female Sprague-Dawley rats
DECISION POINT THREE	
Stage E	Long-term bioassay
DECISION POINT FOUR	

With all of this testing it should be noted that such tests when performed on human subjects are usually designed for the average young adult male. The significant populations of women, the elderly, children, and pregnant females are not tested for some understandable and some not so understandable reasons. The new director of the National Institutes

of Health (a woman) has raised the consciousness of drug testers to these groups and their needs.

All decision points include the evaluation of the cumulative data to that point. At this, the final point, a final evaluation must be applied to the health risk analysis.

C. Risk Assessment

The process of risk assessment is quite complex and can be more fully appreciated at the end of the first third of the course. There have been many good articles and books written over the last ten years which present various points of view on perceived versus actual risk.

IV. TERMS AND NAMES

A. Terms

Prescription Drug - also called an ethical drug. These medications must be prescribed by a licensed physician, dentist or veterinarian. They may be covered by a patent or be in the public domain.

Nonprescription Drug - also referred to as an **Over-The-Counter drug (OTC)**. Sometimes these materials are called proprietary but the latter term can also be applied to patented prescription drugs.

Generic Drug - a drug whose patent has expired and is in the public domain. It is these substances which are now subject to ANDAs. The ANDA must ensure that the proposed medical use for the drug, its ingredients, the strength of the formulation, labeling information, and dosage forms are the same as for the formerly patented drug. The company requesting to manufacture the generic drug must also file test results which show bioequivalency. (see http://www.fda.gov/cder/ob/docs/preface/fp19pref.htm#Therapeutic Equivalence-Related Terms)

IND - investigational new drug

NDA - new drug application

B. Naming

There are three types of names which almost every drug and registered toxin possesses: its chemical name, a generic name, and a tradename.

1. **Chemical name** - the IUPAC or common nomenclature chemical title.

2. **Generic name** - also known as the United States Adopted Name (USAN) - something less tongue-twisting and shorter than the chemical name.

3. **Tradename** - a patented, Madison-Avenue, snappy, memorable ditty - there may be tens of these names depending upon the company, country, etc.

Table 3 lists the types of names for an antidiabetic drug and for a common insecticide.

Table 3 Naming for Drugs & Poisons

	Antidiabetic Drug	**Insecticide**
Chemical:	1-[p-[2-(5-chloro-O-anisamide)ethyl]phenyl]-sulfonyl]-3-cyclohexylurea	O,O-diethyl O-p-nitrophenyl phosphorothioate
Generic:	Glyburide	Parathion
Tradenames:	Adiab®, Diabeta®, Daonil®, Euclamin®, Euglucon®, Gilemal®, Glidiabet®, Hemi-Daonil®, Lisaglucon®, Maninil®, Micronase®, Semi-Euglucon®	Alkron®, Alleron®, Aphamite®, Etilon®, Folidol®, Fosferno®, Niran®, Paraphos®, Rhodiatox®, Thiophos®

C. Drug Ratings

1. Alphabetical ratings pertain to therapeutic value. A class A drug has significant, effective value.
2. Numerical ratings indicate the unique nature of the drug. The lower the number the more unique.

Thus a Class IA drug would be an extremely effective, unique medication.

FUNDAMENTAL PRINCIPLES

IV. ABSORPTION, ADMINISTRATION, DISTRIBUTION, AND BIOTRANSFORMATION

Before discussing the actions and effects of specific drugs and poisons, we have to appreciate the physical and chemical processes to which these substances are subjected when introduced into a living organism. The absorption, distribution and storage, biotransformation, and subsequent excretion of a chemical will determine how and where it should be administered, the extent of its overall effects, and how much of the drug should be given (dose) in order to achieve the desired effect. We should all be able to appreciate the concept of side effects and the methods used to alleviate or prevent unwanted side effects.

A. Absorption

The route which a drug or poison follows to gain entry into an organism are varied. No matter the route, usually one or more biological membranes will have to be penetrated as the substance finds its intended or accidental target(s). A substance can be also be designed so that it will not pass a specific barrier. For example, there are cremes and lotions which are meant for topical (surface) use only. In this section we will review the concept of membranes, discuss membrane permeability to natural and nonnative (xenobiotic) chemicals, and consider how drugs may be designed or formulated for accessibility to an organism.

xenobiotic

1. **Membranes**

A membrane is a semipermeable barrier whose function is to compartmentalize metabolic processes, maintain pH differences on either side, control osmotic pressure and ionic gradients, provide a surface or environment for the stabilization of active biomolecules, provide tissue discrimination, and allow selective access as well as egress to specific metabolites.

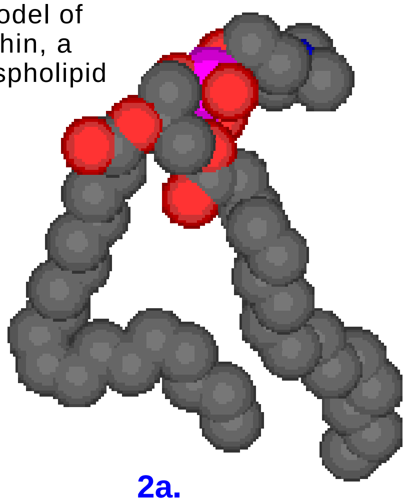
The biochemical structure of a membrane is that of a lipid bilayer composed of phospho- and sphingolipids, as well as cholesterol. These lipids are amphipathic in nature, that is, they each have a polar and a nonpolar end. In water the nonpolar (hydrophobic, lipophilic) ends will seek to avoid the polar solvent and aggregate into a bilayer with the polar (hydrophilic, lipophobic) ends oriented towards the outside of the bilayer. As this structure extends in all directions the exposed nonpolar regions will close up and form a sphere (or ellipsoid) with water trapped inside and excluded outside. See Figures 2a and 2b.

Since the phospholipids have cis-unsaturated fatty acid components there is a certain amount of fluidity to the bilayer. You can picture a sea of lipids moving in a transverse motion across the inside and outside faces. Note that the exterior of the membrane is composed of the polar portions of the biolipids while the interior of the membrane is highly nonpolar. Therefore while there is transverse fluidity there is little or no flip-flopping and exchange of interior lipids for exterior lipids because the polar ends will not easily cross the highly nonpolar interior.

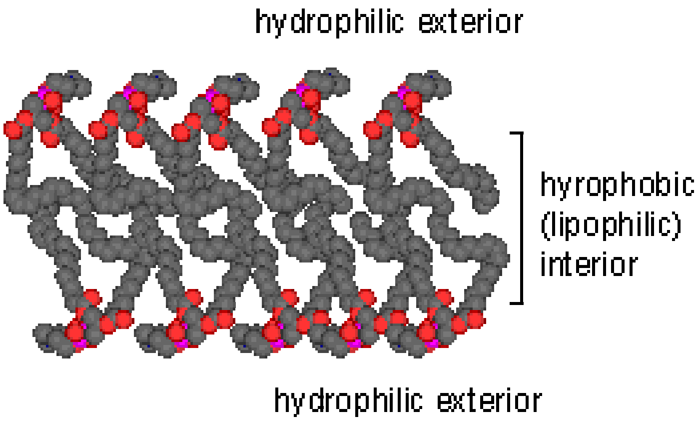
In general the sphingolipids are located on the exterior face of a membrane while the phospholipids make up the inner face. This is understandable when we recall that sphingolipids include the gangliosides and cerebrosides whose polar ends contain carbohydrates or complex carbohydrate derivatives. The large number of chiral centers in carbohydrate molecules offer a complex pattern on the surface of the membrane which can impart a large degree of specificity for a particular cell type. The composition and limited fluidity of the bilayer make the entire membrane asymmetric, that is, different on the inner and outer layers or leaflets.

The semipermeability of the bilayer is evident when we again consider its highly nonpolar interior. Only nonpolar molecules will be able to cross this lipophilic barrier by a simple diffusion process. See Table 4.

A model of lecithin, a phospholipid

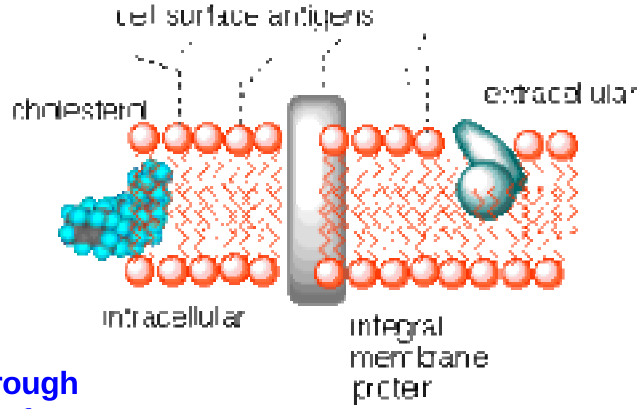


2a.



2b. a lipid bilayer

Figure 2



2c. A very rough schematic of a membrane

2. Membrane Transport

It is easy to see that nonpolar, lipophilic molecules will easily cross membranes. But we know that polar metabolites and ions must also gain entrance to as well as exit from cells and organelles. This is accomplished through another type of membrane component - membrane proteins. These proteins may lie on the surface of the membrane (peripheral proteins) or be located either entirely within the nonpolar interior, or be partially exterior and interior, or completely span the bilayer (integral proteins). See Figure 2c. These proteins function as channels, carriers, receptors, or signal transmission devices.

The overall picture of a membrane then becomes one in which the surface presents a background of lipids in- and onto which are placed proteins with specific positions and functions. This is called the fluid mosaic model as proposed by Singer and Nicolson.

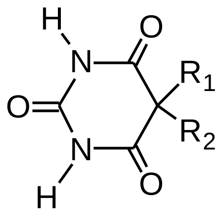
Transport across the membrane may be passive or active.

Table 4

A. Lipophilic, diffusable materials

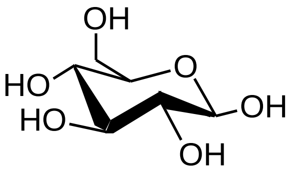
nonpolar gases	N_2 CO_2 O_2 N_2O	
anesthesia gases	$CH_3CH_2OCH_2CH_3$	diethyl ether
	$CF_3CHBrCl$	halothane
central nervous system depressants	CH_3CH_2OH	
	ethanol	

barbiturates

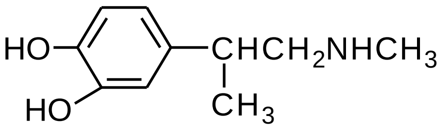


B. Lipophobic substances

Na^{1+}, K^{1+}, Ca^{2+}, Cl^{1-} ions



glucose



epinephrine

a. Passive Transport

Passive transport requires no overt energy expenditure because the substances moving across the membrane are going from an area of higher concentration to one of lower concentration. The two types of passive transport are simple diffusion (osmosis) and facilitated diffusion. Molecules which may undergo simple diffusion can be found in Table 4. They are

highly lipophilic. Less lipophilic (more hydrophilic) molecules must be helped across by membrane proteins (channels, translocases, permeases, symports, antiports, etc.); this is facilitated diffusion or transport. As with enzymes, these membrane proteins have a limited capacity to transport metabolites and can become "saturated". This factor can be of great mportance in drug transport in that some drugs are similar to natural channel "substrates" and can compete with the natural material for transport.

b. Active Transport

This must obviously be the opposite of passive transport. Active transport does require energy, usually in the form of the consumption of ATP or GTP, because the molecules are moving against the concentration gradient from an area of lower concentration to an area of higher concentration. The most well known active transport system is the Sodium-Potassium-ATPase Pump (Na^{+}- K^{+}/ATPase) which maintains an imbalance of sodium and potassium ions inside and outside the membrane, respectively. See Figure 3.

Other active transport systems include the sodium-hydrogen ion pump of the GI tract (stomach) and the calcium ion pump which helps to maintain a low concentration of calcium in the cytosol.

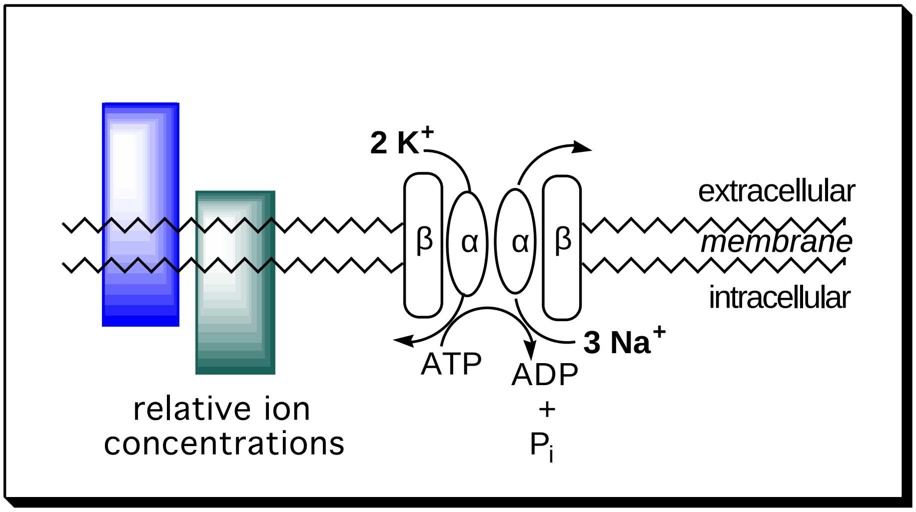


Figure 3
Sodium-Potassium ATPase Pump
An Active Transport System

3. **Effects of pH on the Passage of Molecules Across Membranes**

As you can see a molecule must be quite nonpolar or lipophilic in order to easily diffuse across a membrane. If a substance has a large number of polar groups or is in a charged state it will not pass by diffusion. However, there are molecules which have conjugate acid-base forms, one of which can be uncharged. The pH of the environment will determine the form of the species in question, conjugate acid or conjugate base, charged or uncharged.

The pK_a of the molecule is the pH at which there is a 50:50 mixture of conjugate acid-base forms. The conjugate acid form will predominate at a pH lower that the pK_a and the conjugate base form will be present at a pH higher than the pK_a.

$$HA \rightleftharpoons H^+ + A^-$$

conjugate acid	conjugate base
(uncharged) **lipophilic**	(charged) ***lipophobic***

$$HM^+ \rightleftharpoons H^+ + M$$

conjugate acid	conjugate base
(charged) ***lipophobic***	(uncharged) **lipophilic**

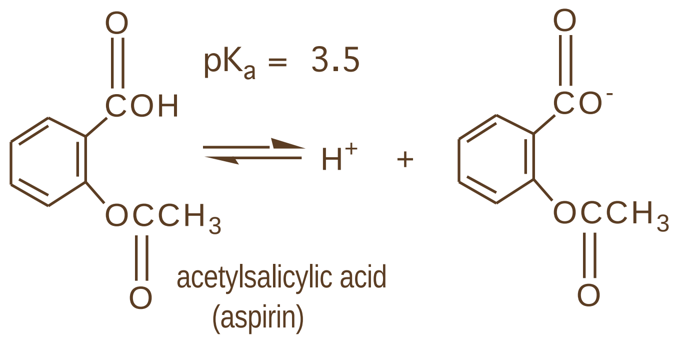
The Henderson-Hasselbalch Relationship

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

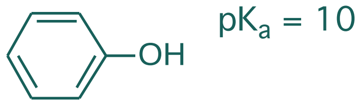
$$pK_a = -\log K_a$$

$$pH = pK_a + \log \frac{[base]}{[acid]}$$

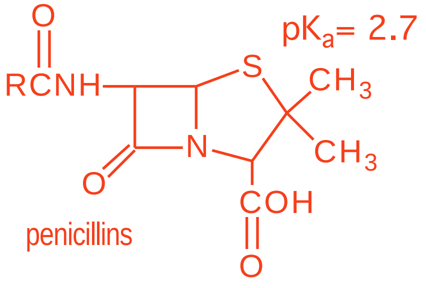
This concept can be applied to the ionized states of drugs and poisons in various body fluids. The gastrointestinal (GI) tract is the most common route for the introduction of xenobiotics into the body. The mouth has a pH of 4-5, the stomach about 2, the small intestine 5-7 and the large intestine about 8. Consider the ionized states of the molecules below and determine where they might be absorbed in the body.



acetylsalicylic acid
(aspirin)



phenol(used in removing
skin for a form of"facial")



penicillins

4. **Specialized Membranes**

Read Goldstein and Betz "The Blood-Brain Barrier" **Scientific American** September 1986

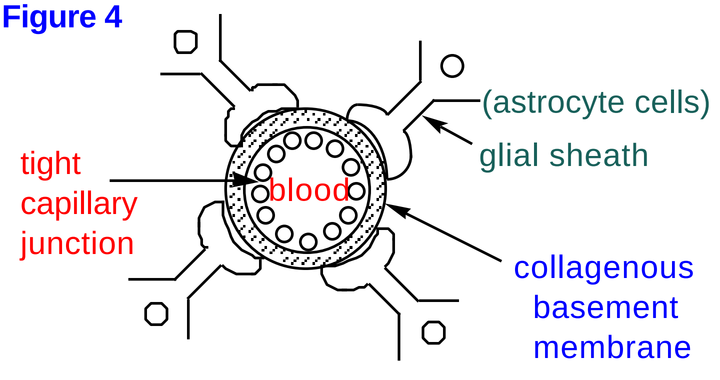
a. The blood-brain barrier (BBB, B^3)

The brain is the control center of the body. Although it comprises only 2% of the body mass by weight, it uses 16% of the resting bloods supply requiring ten times the blood flow that the rest of the body needs. Because of its importance to life it is protected from physical trauma by the bony structure of the skull on the outside and from chemical trauma by the highly selective blood-brain barrier from within.

Thoughout most of the body the tissues and cells are separated from the blood by a thin lining of capillary endothelial cells. In the brain these cells are packed much closer together than in the rest of the body forming a tight junction. Surrounding the tight junction of endothelial cells is a collagenous basement membrane which is fairly impervious to many materials. Recall that the protein collagen of which our skin, bone, teeth, and ligaments are constructed has a unique composition and structure. With almost 60% of its amino acids as glycine and proline, it forms a triple helix stabilized by hydrogen bonding from one strand of the helix to another. In addition, carbohydrates can be covalently attached to the amino acids of the triple helix and the helices can be chemically crosslinked for strength and elasticity.

Added to these two layers is an additonal barrier formed by the brain itself. Foot-like projections of brain cells called astrocytes encase the collagenous basement membrane forming a glial sheath. These cells are believed to "induce" the formation of the tight junction of the endothelium. In addition the endothelial blood cells and astrocytes contain enzymes which can alter an invading molecule's structure. See Figure 4. This triple barrier allows the brain to be very selective in the transport of metabolites and xenobiotics into its domain by means of facilitated diffusion and active transport.

Figure 4



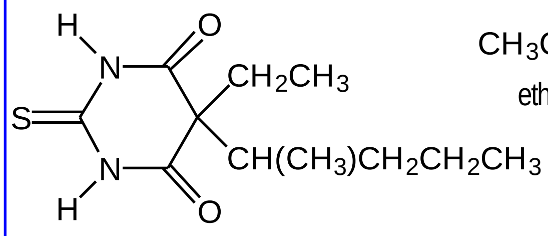
You can read more about the glial sheath in Kimelberg and Norenberg's article "Astrocytes" **Scientific American** April 1989. Much of this paper will be more relevant when we discuss the nervous system.

Once again we find that the more lipophilic a molecule is, the more easily it can enter the brain. This is most evident when you look at the structures of general anesthesia gases and central nervous system (CNS) depressants.

General Anesthesia Gases

N_2O nitrous oxide	$CF_3CHClBr$ halothane	$CHCl_2CF_2OCH_3$ methoxyflurane
$CHF_2CF_2OCHF_2$ enflurane	$CHF_2CHClOCHF_2$ isoflurane	

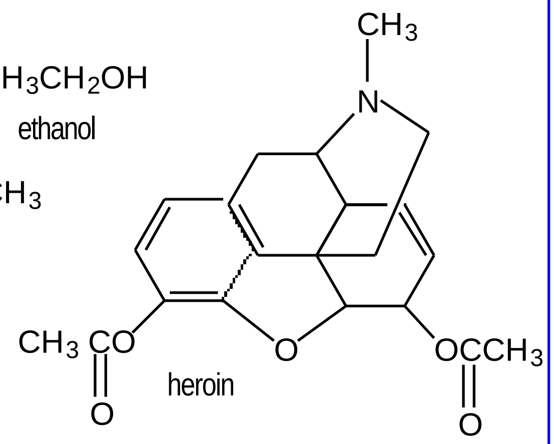
CNS Depressants



thiopental

CH_3CH_2OH

ethanol



heroin

The exterior of the brain and spinal cord is cushioned within the skull and backbone by the cerebral-spinal fluid (CSF). The CSF is kept from direct contact with the brain by the arachnoid membrane covering the surface and the choroid plexus, tissue which selectively interacts with the contents of the CSF. An injection into the spinal cord or through the skull can put a drug directly into contact with the choroid plexus and the brain. For more on this subject you can read "The Mammalian Choroid Plexus" by Spector and Johanson **Scientific American** November 1989.

The permeability of the blood-brain barrier can be changed by disease states such as encephalitis or meningitis or it may be temporarily disrupted by introducing the drug in a hypertonic solution of a solute, for example, glucose. The difference in osmotic pressure will cause the endothelial cells to lose water and shrink (crenate) thus loosening the tight

junction. This method has been used to introduce the enzyme hexoseamidase A into the brains of test animals.

Hexoseamidase A is the enzyme which is deficient in Tay-Sachs disease. This inherited condition results in an accumulation of GM_2 gangliosides (sphingolipids) in the brain. The consequences are mental retardation, paralysis, blindness and death by the age of 3-4 years.

Still another experimental route to introducing otherwise excluded molecules into the brain is to chemically modify them so that they are lipophilic and therefore can passively diffuse. The brain, just as most other organs and tissues of the body, has enzymes to metabolize or biotransform metabolites in order to use and then get rid of them. Many of these pathways are oxidative. A reduced species or derivative which is lipophilic can enter the brain by simple passive diffusion there to be oxidatively transformed into an active state. Compounds which have been tested in animals include derivatives of 2-PAM (an antidote for organophosphate insecticide poisoning) and phenylethylamine (similar to amphetamine type molecules). Figure 5 illustrates the general concept behind this method.

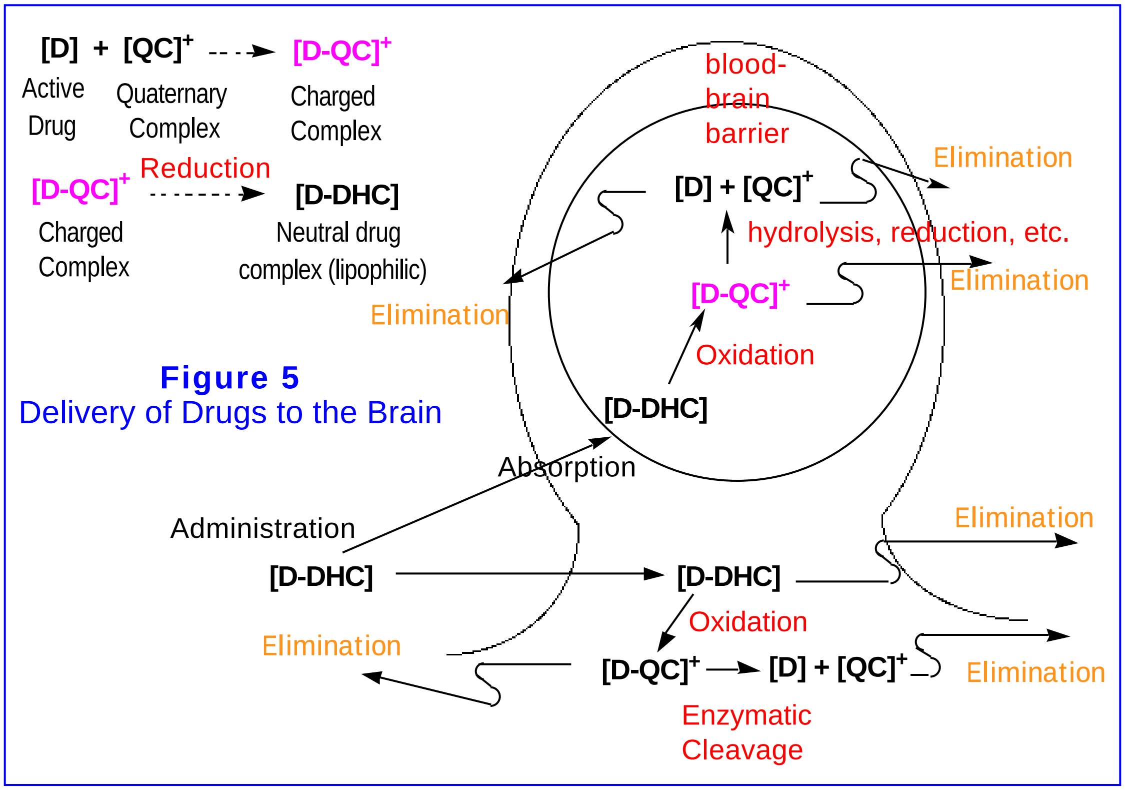
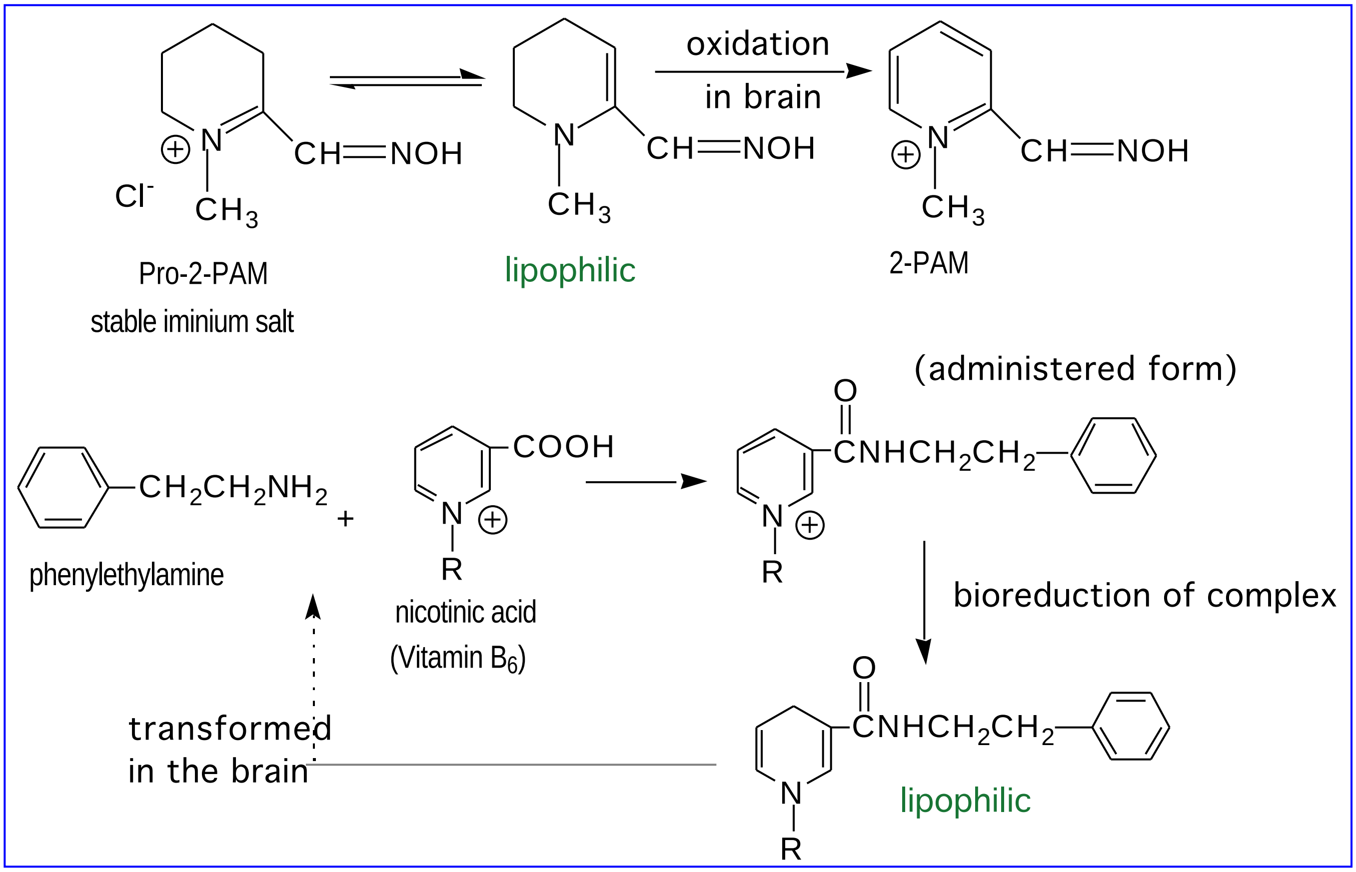


Figure 5
Delivery of Drugs to the Brain

b. The Placental Membrane

The placenta is an extremely permeable membrane which separates the fetal blood supply from the maternal blood supply. Normally it allows for the exhange of gases, nutrients and metabolites between mother and child. It



takes about 40 minutes for a drug to reach an equilibrium across the placental membrane so that the fetus will be affected. Evidence of this can be seen if a mother is given a barbiturate during childbirth. When administered within 15-20 minutes of the actual birth, the baby will be awake and alert. However, if the sedative is administered 2-3 hours before actual birth, the newborn will be drowsy. This effect will last for 12 to 24 hours in the neonate.

Nowhere are the effects of the permeability of the placental membrane more graphic than in the incidence of fetal alcohol syndrome (FAS) in the children of alcoholic mothers. FAS produces distinctive anatomical features and mental retardation. Ethanol is called a teratogen because it causes genetic malfunction. Recall that thalidomide was a teratogen.

We are also well aware of the addiction of newborns to heroin if their mothers were took the drug during pregnancy. So-called "cocaine babies" are restlessness, nervous and irritable. Fetal nicotine effects can be seen in low birth weight neonates. We are still not sure about the teratogenicity of heroin and cocaine. The latter can cause abortion and so could very well be teratogenic.

B. ADMINISTRATION OF DRUGS (ROUTES OF EXPOSURE FOR POISONS)

Keeping in mind, then, that lipophilicity is highly important to the absorption of a drug or poison through the membranes of the body, we can now proceed to a discussion of the common routes used for the administration of drugs and those routes by which poisons find their way into an organism. The site of the desired or undesired effect must be considered as well as whether that effect should be localized to the immediate area of application (topical) or whether it can be systemic, that is, spread throughout the body. Recall that the blood itself is buffered at a pH of about 7.4. Also remember that the body is 70% water by weight.

The general factors influencing drug administration are:

1. solubility (oils are more difficult to administer than water)
2. rate of dissolution (this includes the physical state of the xenobiotic)
3. blood circulation to the site of administration (heart - high; adipose - low)
4. surface area of the site of application
5. concentration of the xenobiotic
6. amount of time that the material stays at the site

We will use these factors as we consider the major routes of administration and exposure.

1. Ingestion (Gastointestinal Tract)

a. Mouth - The mouth or buccal area has a pH of 4-5. The surfaces which can absorb chemicals include the tongue (especially under the tongue) and gums. The terms used for top and bottom of the tongue administration are superlingual and sublingual, respectively. Nitroglycerin, a potent vasodilator, used to be given for angina (heart pain) exclusively by sublingual tablets. The large advantage to sublingual administration is a faster route to the heart itself via the blood circulation. Today nitroglycerin can also be applied via skin patches (transdermal) and superlingual sprays.

b. Stomach - The stomach maintains a pH between 1 and 3 through the secretion of hydrochloric acid (HCl). The absorption of materials from this acidic environment depends upon their lipophilicity at that pH, the bolus of food or liquid present in the stomach at the same time, and the emptying time of the stomach contents into the small intestine. Lipophilic molecules such as ethanol and uncharged conjugate acid forms such as that of aspirin can be absorbed.

c. Small intestine - Most materials are absorbed in the small intestine. Not only is the pH such that neutral forms will exist and so be lipophilic, but also the absorptive surface area of the small intestine is immense. There also exist transport systems for

intestinal absorption which are meant for nutrient molecules but could certainly be fooled by xenobiotics with similar structures. Because of this almost indiscriminate absorption process, materials will vie with each other for passage. pH will be a factor as will the food and liquid content of the space, the effects of the intestinal digestive enzymes, and the molecular sizes and shapes of everything present to be absorbed.

2. **Inhalation**

a. Nose - The nasal mucous membranes are absorptive surfaces which line the passage to the rest of the airway. Mucous, a mixture of proteoglycans, acts as a protective coating and lubricant to remove unwanted particles. When the membranes become irritated they will swell. Decongestants are used to constrict the swollen blood vessels.

This route is currently being clinically tested as an application method to deliver insulin for the treatment of diabetes. The major problems in using drugs in nasal aerosols are finding the proper surfactant to breach the mucous membranes and avoiding excessive irritation of those membranes.

b. Mouth - The mouth is a passageway to the lungs as well as to the stomach.

c. Nasopharynx (throat) - Cilia line this part of the tract. They are part of a sensory system which eventually cause reflex actions such as sneezing and coughing.

d. Bronchi

e. Lungs (alveoli) - The lungs present a large surface area for absorption, about 100 meters^2. They, as well as the pathway before them, also contain cilia, tiny hairlike projections which can help to sense and trap particles as they travel through the airways. Stimulation of the cilia causes a sneeze or cough reflex meant to dislodge the particle. If the particles are too small and finely dispersed to be sensed and expelled, they can cause serious damage to the surface of the lungs. Asbestos (asbestosis), silicon (silocosis), cotton dust, coal dust (blacklung), and sugar cane pulp all present serious irreversible damage to the lungs due to occupational exposure to the fine dust. These materials accumulate in the lung because they cannot be absorbed through the lung membranes due to their physical states. Substances of molecular dimensions are fairly easily absorbed by the lungs while particulates are not.

This route presents not only a quick, efficient way to deliver drugs into the bloodstream but a vulnerable route for poisoning. In early 1989 the FDA gave "Treatment IND" status to an aerosol form of pentamidine for the prevention and treatment of ***Pneumocystis carnii*** pneumonia which is the most common life-threatening infection seen in AIDS patients.

3. **Skin**

The skin of an average adult presents a surface area of about 18,000 cm^2. It is a multi-layered collagenous complex(epidermis, dermis) which is relatively impermeable to most materials. However, given the right conditions of solvent and specific skin surface, the skin presents a very accessible route for drug application. Some of the latest research and development (R&D) efforts of major pharmaceutical firms has been in the area of transdermal applications.

Nitroglycerin was marketed for a while in an ointment form for application to the forearm surface. The ointment consisted of a base of petrolatum, the drug, and lactose, which increased the drug's stability and reduced its explosiveness. If it was applied to another body part or another person, there could be some very unpleasant side effects.

The most current method of nitroglycerin application is a transdermal device or skin patch. A cross section of such a patch is illustrated in Figure 6. The patch is actually a multi-layered polymer stack. The semipermeable membrane which comes in contact with the skin is usually composed of an ethylene-vinyl acetate copolymer or polypropylene. The reservoir contains the drug in a hydrogel or polymer matrix or solvent (the material must be chosen to insure uniform delivery). Examples of some solvents used include dimethyl sulfoxide (DMSO), sodium lauryl sulfate (SDS - a detergent) and propylene glycol/oleic acid.

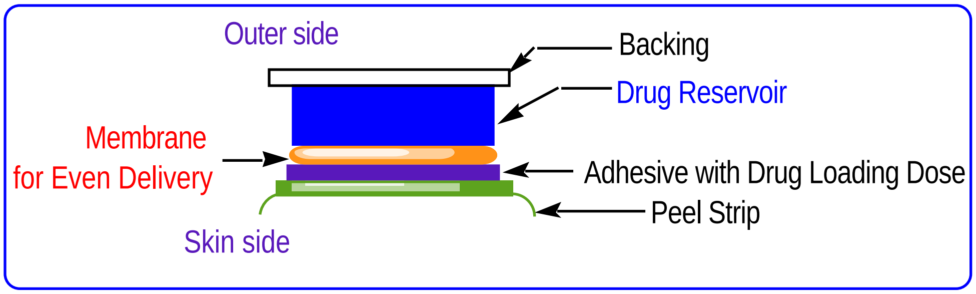


Figure 6
Transdermal Devices

This method has been patented as a Transderm V® system and several drugs have been marketed using it.

Transderm® Nitro (CIBA), Nitrodisc (G.D. Searle), Nitrodur (Key), Minitran (3M)

The size of the patch can be varied to deliver different doses of nitroglycerin (0.1, 0.2, 0.4, 0.6 mg/hr)

A comparison of the duration of effects of the various nitroglycerin administration methods showed that sublingually the duration was 30 minutes; tablets, 12 hours; transdermal, 24 hours.

Transderm® Scop (CIBA) - Scopolamine for motion sickness; effective for 3 days; delivers about 5 μg/hr.

Other types of drugs using this delivery system are clonidine and timolol (antihypertensives),estradiol (postmenopausal prophylaxis), fentanyl (opiate analgesic), and nicotine ("the patch").

When studying dermal absorption using animals, a species with an absorption comparable to that of humans must be used. For example, rat and rabbit skin are more permeable than human while that of cats, dogs, and mice are less permeable. Guinea pigs, pigs and monkeys have dermal characteristics similar to those of humans.

Pesticides are usually applied in some type of solvent that will minimize the insect's waxy and chitinous protective barrier. Sometimes the pesticide itself is more selective for one type of barrier than another. DDT is less easily absorbed through the skin than through the chitinous exoskeleton of an insect.

4. More parenteral (other than through the intestine) routes of administration

a. Rectal - This method is used for antiemetics (to stop vomiting - emesis).

b. Vaginal - This is a good route for intrauterine contraceptives such as Progestasert® (Alza Corporation). This progesterone contraceptive is applied via a T-shaped unit which is made up of the drug in silicone oil, an ethylene/vinyl acetate copolymer, barium sulfate and titanium dioxide. It delivers 65 micrograms of drug per day for one year.

c. Ocular - Drops are a common form of topical delivery of drugs to the eye but frequently the drug can be washed away by the tears formed as a reflex to the material being infused. Ocusert® is a form of pilocarpine which is used for the treatment of glaucoma by insertion onto the inner portion of the lower eyelid.

d. Sublingual - Already mentioned for nitroglycerin, this route is one of the quickest to the heart (see Figure 8).

e. Subcutaneous (s.c.) (beneath the skin). Norplant® is an s.c. form of the progestin levonorgestrel, a contraceptive. Approved in 1991 for use in the United States, it consists of six small capsules of an elastomer containing the drug which are implanted beneath the skin of the upper arm under local anesthetic in the physician's office. This method has been found to produce effective contraception for up to five years. A modification uses two 4-cm solid rods and has effects up to three years.

f. Sublesional

e. Intra-
arterial, articular (joints), cardiac, dermal, lesional, muscular (i.m.), peritoneal (i.p.), spinal (-thecal), venous (i.v.).

Intravenous injection is the quickest common pathway to introducing a drug and having it reach its target within an extremely short time period. This relates to the "rush" of drug effects experienced by i.v. drug abusers. It is valuable for emergency use but unwanted side effects may also be intensified.

Intraaraticular injections of cortisone are used to alleviate the pain from osteoarthritis, especially in the knee, elbow and hands.

Intramuscular and subcutaneous routes deliver the drug promptly, if water-based solutions are used, or slowly, if other types of solutions are used. They are better for larger volumes of material although local irritation can result.

f. Hypodermoclysis - into subcutaneous, loose tissues such as the thighs, buttocks, breasts, under loose skin. This method can be used after shock, hemorrhage or diarrhea when large volumes of fluids might have to be restored quickly. The temperature of the materials to be injected as well as ionic strength are very important.

5. **Other Modes of Drug Delivery Including Some Which Are Experimental and Promising**

a. Monoclonal antibodies are homogeneous proteins related to the antibodies which are the products of our body's own natural protective chemistry, the immune system. They can recognize particular chemical groupings and so are extremely tissue-specific. Researchers have chemically linked toxins to antibodies and targeted them for cancer tissue, for example. These could be the pharmaceuticals which are the closest yet to Ehrlich's "magic bullets".

b. Liposomes - Colloidal vesicles can be used to deliver drugs. They may be composed of synthetic polymers, proteins carbohydrates or

lipids. Liposomes are synthetic multilayered phopholipid vesicles which can contain medications. They have a reasonable lifetime in the bloodstream and very slowly release their contents. These containers can be constructed so as to release their contents upon a signal from an external source. Liposomes can also be linked to monoclonal antibodies and thereby become tissue-specific.

c. Proteins and peptides used as drugs require special modes of delivery because of their molecular sizes and susceptiblity to digestion. They can be linked to endogenous carriers such as vitamin B_{12} or can form complexes such as an insulin-enzyme-albumin conjugate which will go wherever there are insulin receptors.

d. Pumps can be used such as the Infusaid® which is a device which designed for the steady infusion of insulin to diabetics. The ultimate in this delivery mode would be a glucose-sensitive self-modulating system.

Pills can operate as osmotic "pumps" if they have miniscule laser-drilled holes in them. The pill coating is selective in that water from the body is allowed to enter to displace the drug at a constant rate.

e. Pulsatile Polymeric Controlled Release Systems can be controlled by electromagnetic fields external to the body.

f. Electroporation involves passing a low current of electricity across the skin which increases its permeability.

g. Drugs are adsorbed onto ion exchange resins and are displaced by appropriate ions present in the GI tract. Since the GI ion concentration is fairly stable the rate of drug delivery is steady and controlled.

h. Implantable machines may be employed such as Port-a-Cath® which is a metal chamber which can be implanted under the skin of the chest cavity (also within the cranium) and has a catheter which can deliver drugs directly into a blood vessel (or onto the surface of the brain).

i. Erodable or biodegradable polymers can be mixed with drugs in order to deliver them slowly and effectively. pH-sensitive erodable polyorthoesters are already undergoing trials.

Veterinary methods could also include intra-ruminal, mammary, thoracic, and epidural administration.

And who could forget those tiny time-capsules - coated and timed-released methods already in use.

6. **Considerations**

a. Drug Administration - The principal concern when prescribing a self-administered drug is patient compliance. Is the medication convenient to take? How frequently must the patient take it? What other medications are being taken? What is their form of delivery? These factors usually make the oral route the one most used. Following this in close order are dermal and subcutaneous methods as well as nasal and bronchial aerosols.

b. Poison Exposure - Since no one wants to be subjected to poisons our considerations here are different. We have to look at the most common means of exposure. Toxicity will vary depending upon the route taken. As you might expect dermal absorption, inhalation and then ingestion are probably the most frequent paths of entry by toxins into the body. A poisoner would also use one of these three routes if he/she could not intrude into his/her victim's blood supply directly. Note that snakes, spiders, and insects bite or in some other way puncture their victims and so employ an intravenous, intramuscular or subcutaneous route.

C. The Distribution and Storage of Drugs and Poisons Within the Body

The body is composed of about 70% water by weight. The fluid portion of the blood (plasma) accounts for 6 liters or about 4% of that total. Intracellular water constitutes about 41% while interstitial (between cells) fluid is 13%. The rest is extracellular. Therefore solubility in water will be an important factor in drug distribution. Fat depots within adipose and other fatty tissues are also important in that they can sequester lipophilic molecules and release them very slowly back into the general blood circulation. This is the case with tetrahydrocannabinol (THC), the psychoactive component of marijuana. It shows two periods of presence in the blood; the first immediately after consumption and the second a more lingering presence for up to 27 days after use.

The cardiac output or flow of blood normally is so rapid that the distribution of a drug or poison throughout the body is complete within a short period of time. An entire 6 liter supply of blood is pumped through the body at the rate of about once per minute. Some organs and tissues are more highly perfused with blood than others, such as the brain, heart, liver, and kidneys. Adipose (fat) tissue is not as richly endowed. Should a person be in shock or have suffered a myocardial infarction (heart attack), however, the cardiac output can be sharply diminished and a route of drug administration normally used may be circumvented because of poor

distribution in order to get as close as possible to the organ or tissue requiring treatment.

1. **Sites of concentration** - There are other sites where drugs may accumulate besides in the water portion of the blood or within fat tissue. The blood also contains a host of proteins which can bind vitamins, metabolites and xenobiotics. In addition, the collagen network of bone and connective tissue can act as a depository for various ions.

a. Plasma proteins (PP) - Blood plasma is composed of water and a larger number of proteins which are instrumental in metabolite transport and the immune system. Called the plasma proteins, they can interact through noncovalent interactions(hydrogen bonds, salt bridges, hydrophobic interactions) with the materials being transported in the circulation, including drugs and poisons. An equilibrium is established between the free drug and protein and that which is bound. It is the free form of the drug which will be available for absorption into a cell.

$$\text{Drug} + \text{PP} \rightleftharpoons \text{Drug-PP complex}$$

PP - plasma proteins

Serum albumin is the main protein constituent of the PPs. With a molecular weight of about 66,000 daltons, it serves to maintain the osmotic pressure of the blood and to transport materials throughout the system. Figure 7 illustrates a densitometer readout of an electrophoretic separation of PPs at pH 8.2.

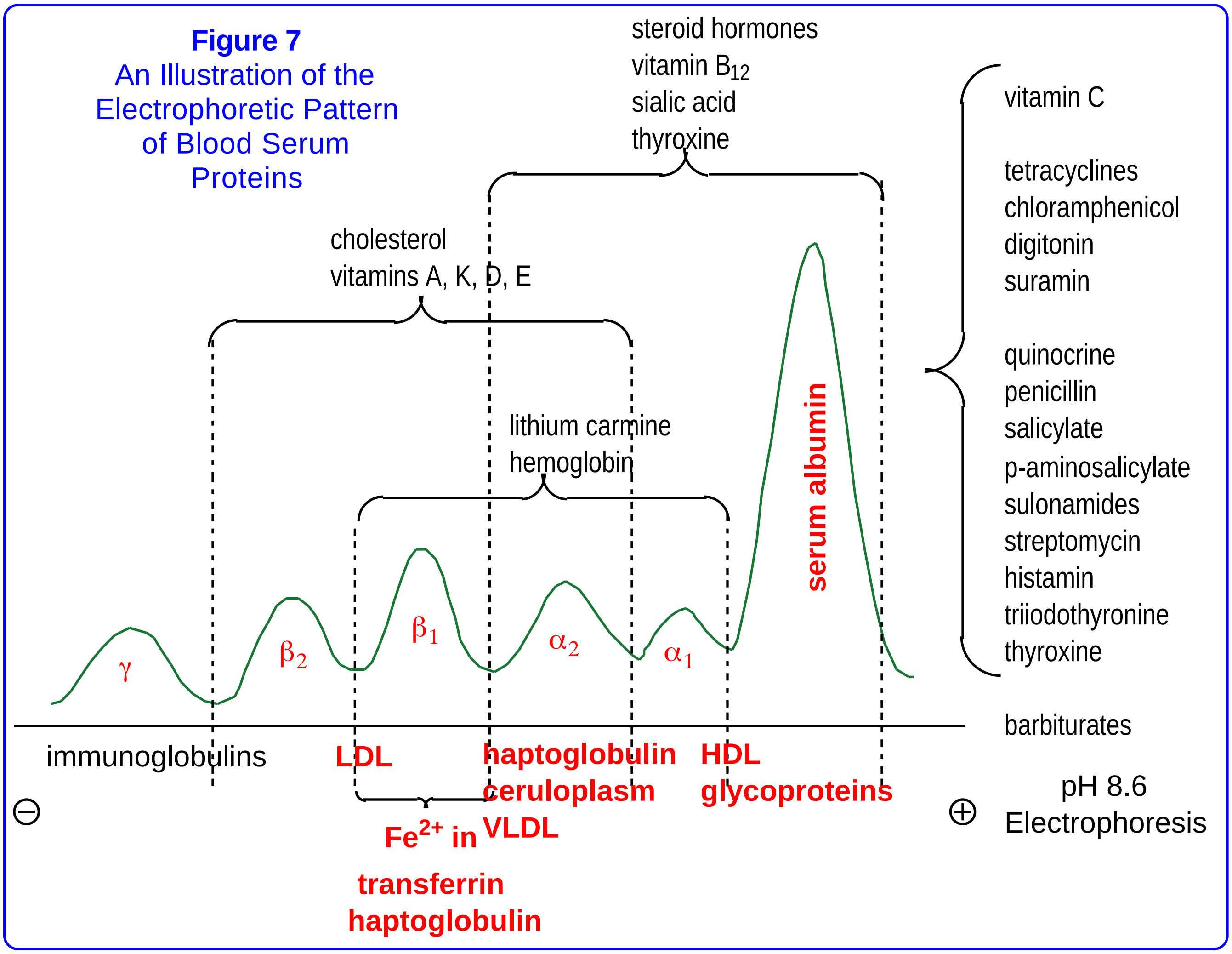
As you can see all of the major protein bands are negatively charged at that pH which in itself would mean that the possibility of electrostatic attractive forces could exist between the proteins and positively charged molecules in the blood.

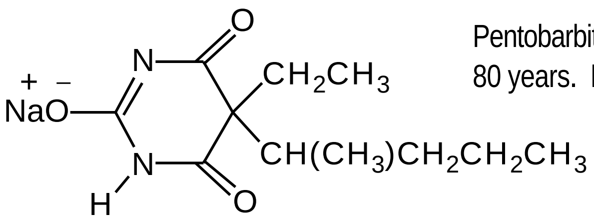
Binding with PPs can prolong the lifetime of a drug and its effects within the body. There can also be competition for binding with PPs. Drugs can displace metabolites (food molecules, vitamins) and vice versa. One drug can displace another if the former is more tightly bound. These factors, including diet and lifestyle, must be considered in prescribing drugs, especially when prescribing combinations of drugs.

As we age our PPs also get older and become senescent, that is, they do not bind drugs as well as when we were younger. Therefore more drug will be in the unbound form and the dose prescribed for a medication which binds to PPs should be lower for a senior citizen than for a young executive.

Plasma protein binding can be a useful factor in the treatment of various conditions.

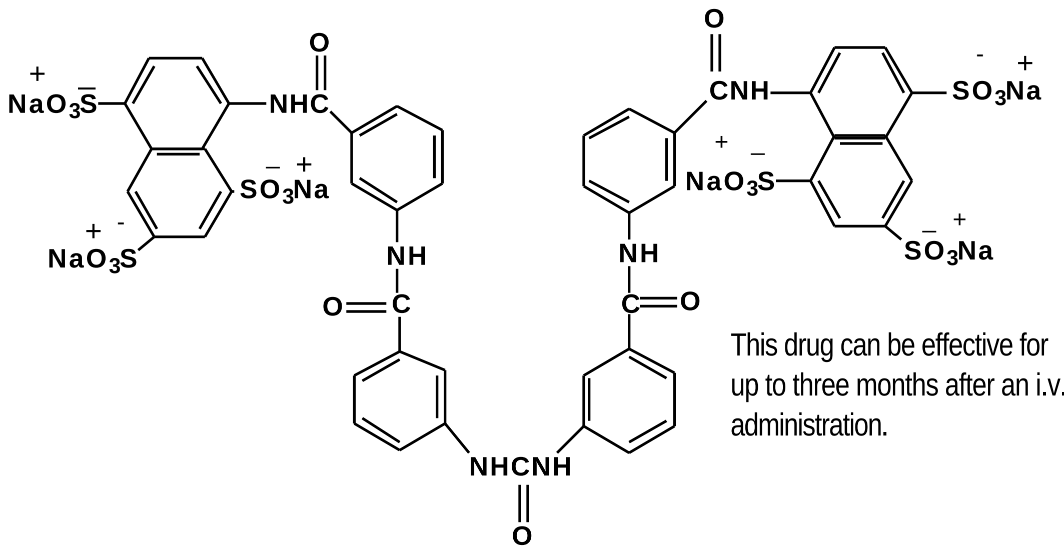
Figure 7
An Illustration of the Electrophoretic Pattern of Blood Serum Proteins





Pentobarbital has a half-life in the blood plasma of 80 years. In fact, it is metabolized in 4 to 6 hours.

The following drug shown is suramin, used for the treatment of sleeping sickness which is caused by the protozoan ***trypanosomiosis.***



This drug can be effective for up to three months after an i.v. administration.

b. Bone and connective tissue - Collagen and elastin are the protein components of bone and connective tissue. They are fibrous glycoproteins arranged in three intertwined chains called the collagen triple helix. In bone these helices are not aligned directly but have spaces between them which can be filled with a form of mineral, hydroxyapatite - $Ca_3(PO_4)_2 \cdot (OH)_n$. The spaces can also be filled with other minerals and ions such as Pb^{+2}. Approximately 90% of absorbed Pb will go into bone tissue and be stored there. When the lead is mobilized from the bone and migrates to soft tissue that it can exert its toxic role as an enzyme inhibitor.

Fluoride ion, F^-, replaces hydroxide in the normal hydroxyapatite deposits in bone. This replacement causes a hardening of the teeth and protects them from decay. Too much fluoride will cause mottling (discoloration). Fluoride is toxic but is tied up preferentially in bone and once released has such a low solubility in water that it is not believed to reach toxic levels.

Strontium ion, Sr^{+2}, can replace calcium ion in bone. If a product of radioactive fallout, strontium-90, $^{90}Sr^{+2}$, is placed in bone, however, its radioactive decay will destroy both bone and surrounding tissue.

c. Organ tissues - Some organs can concentrate xenobiotics. Since the liver is the main organ of metabolism, it is understandable that most substances pass through it. The amount of chloroquinine, used in the treatment of malaria, can be 200 to 700 times higher in the liver

ASIDE 1 -GET THE LEAD OUT!

This expression not only relates to getting work done but it also pertains to the detrimental effects of lead in our immediate environment. In 1989 an alarm was sounded when excessive amounts of lead were found in the bones of very young children while their blood levels were in the CDC (Centers for Disease Control) "acceptable" range of 22-26 mg/dl. For a long while it had been thought that if the symptoms of lead poisoning or plumbism (loss of reasoning ability, convulsions, coma, or death) were not evident, there wasn't much cause to worry. However, a recent longitudinal study over 11 years (**NEJM 322** pp. 83-88, Jan.11, 1990) indicates that long term low dose lead exposure without overt signs of poisoning leads to deficits in intelligence, language processing, atttention, and overall classroom performance and causes behavioural problems. Since that those reports there has been evidence of lead leaching from printing on food containers and lead crystal decanters.

Both **Time** (February 25, 1991) and Newsweek (July 15, 1991) had feature articles on lead poisoning in children not only from peeling paint in ancient tenements and leaded gasoline remnants found in the soil of the inner city, but in the remodeled older homes of middle income families. Lead is almost ubiquitous and very insidious in its effects.

Biochemically lead binds to sulfhydryl (-SH) groups in proteins, that is, it can inhibit enzymes and affect the structures and functions of structural proteins. Some of the effects of this binding include increased permeability of the blood-brain-barrier, breakdown of vascular tissue, destruction of the ground substance in cells leading to conditions of anemia and kidney damage.

The CDC standards for acceptable levels of lead in the blood, especially the blood of children, are likely to be changed in the near future to much less than the 25 mg/dl now permitted and our society will be living with the results of insufficient studies for at least a generation to come. Maybe we can avoid the destruction of our civilization in contrast to that of the Roman Empire (one of the theories of its decline involves the lead used in making the aqueducts supplying water to Rome).

than in the blood plasma. Neuroleptic (antipsychotic) drugs such as chlorpromazine are very liver-bound and their maximal serum concentration might not be attained until two weeks of oral therapy have been undertaken. In fact, other agents related to chlorpromazine concentrate in various tissues. Their concentrations in the blood are therefore very poorly correlated with the clinical responses observed.

d. Adipose tissue - As mentioned earlier very lipophilic drugs will be stored in fat tissue. In a person who is chronically obese, up to 50% of his/her weight is lipid. As a comparison, only 10% fat will be found in someone who is starving. What this means is that a higher dose of a particularly lipophilic drug would be needed for someone who is overweight. Should dieting accompany the drug regime, adjustments might have to be made due to the release of stored drugs as fat tissue is diminished.

Fat will store toxins such as the polyhalogenated pesticides DDT, Dieldrin and Aldrin. The now banned plasticizers PCBs and PBBs are also polyhalogenated. It was DDT which caused a problem with certain species of predator birds. The pesticide accumulated in waterways where it was taken in by microorganisms and then by fish

and stored in their fatty tissues. In nature's food chain of predator and prey the DDT eventually made its way into the peregrine falcon and eagle where it interfered with the steroid-directed production of egg shells. The shells were too fragile to sustain the necessary strain of the incubation period and these raptors were in danger of extinction. For the falcon a program of egg removal and human incubation with subsequent return of the fledglings to the nests has worked, even though the original problem is still with us.

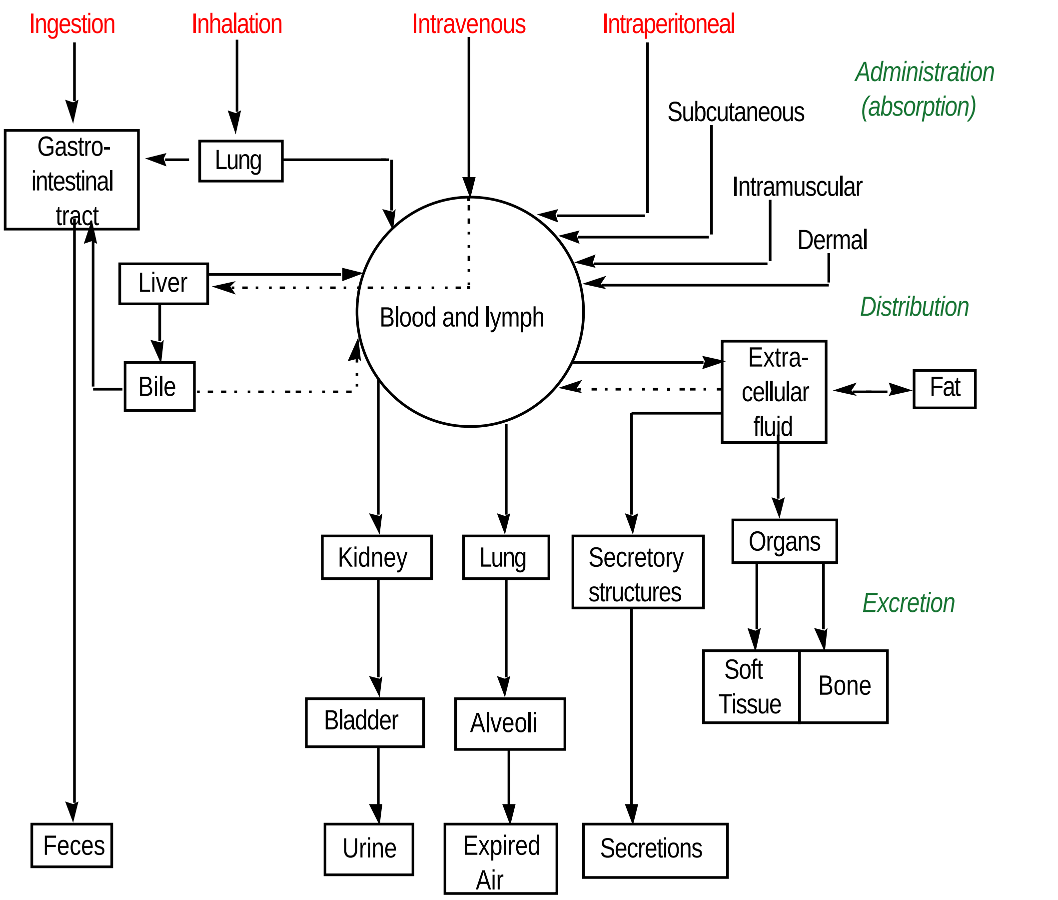


Figure 8

Routes of
Administration, Distribution, Excretion

Adapted from Casarett and Doull, Toxicology.

Modified, and used with permission of Macmillan Publishing Co.

D. Biotransformation

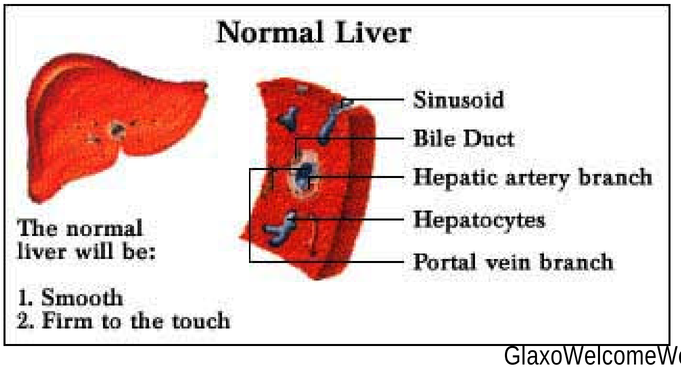
In an example cited previously you saw that pentobarbital had an extraordinarily long half-life if we considered only its binding to plasma proteins. In reality it is metabolized in 4 to 6 hours. This process of metabolism is called biotransformation. All living organisms have developed enzymatic schemes to alter natural and xenobiotic materials for the purpose of eliminating them from the body. Since the major route of elimination for animal species is through the kidneys, most molecules have to be hydrophilic or be converted to hydrophilic species in order to dissolve in the water leaving the body. **Biotransformation is** therefore **the alteration of lipophilic substances to hydrophilic ones for subsequent excretion**. The endogenous molecules which are converted by this means are the steroid hormones, bile acids, cholesterol, neurotransmitters, and vitamins. Those substances not converted will probably be excreted unchanged in the feces.

biotransformation

1. Sites of biotransformation

Biotransformation can occur almost anywhere in the body. However, there are certain organs and tissues which are the primary sites for the processes involved.

a. The Liver - The liver is the largest organ in the body weighing about 3 pounds. It is the major site of metabolism and the formation of bile acids. When foods and other xenobiotics are ingested they pass through the walls of the intestine into the blood and travel via the large portal vein to the liver. Materials can also reach the liver after skin and lung absorption. If the dose of a drug or poison is large, it may take several passes through the liver in order to be completely metabolized.



GlaxoWelcomeWeb Site

The hepatic (liver) intracellular site of biotransformation is the surface of the organelle known as the smooth endoplasmic reticulum (SER). When attempts are made to isolate this organelle by disruption and differential centrifugation, the extensive membraneous structure breaks up into tiny vesicles (like liposomes). These vesicles are called the microsomes or the microsomal fraction of a liver homogenate. When nerve cells are disrupted in a similar manner, they form vesicles known as synaptosomes. As we discuss further the process of biotransformation we will refer to microsomal sites and

microsomes

nonmicrosomal sites, that is, those associated with the smooth endoplasmic reticulum and those not so asociated, repectively.

b. Other sites - As was mentioned before, there are many tissues and body fluids which contain the necessary enzymes for biotransformation. For example, the other fractions of the liver homogenate, the brain (see the Section on the blood-brain barrier), blood plasma, lungs, intestines, kidneys, and the skin all contain nonmicrosomal enzymes which can begin the transformation of lipophilic materials into lipophobic ones.

2. **General Reactions**

The enzymatically catalyzed reactions of biotransformation can be categorized as two general types: nonsynthetic and synthetic (or conjugative). The nonsynthetic are further delineated as oxidation, reduction, and hydrolysis. The role of the nonsynthetic reactions overall is to prepare a molecule for the synthetic pathways by introducing reactive groups. For example, one such reaction might be to convert an aromatic ring (lipophilic) to a phenol so that it might undergo esterification. The usual groups which are produced or introduced are -OH, -COOH, -SH, or NH_2. The nonsynthetic reactions are frequently nonmicrosomal and can occur as a xenbiotic first enters the body or bloodstream.

The synthetic reactions involve the combination of the xenobiotic with another molecule prior to elimination via the kidneys. Since these are synthesis reactions they require energy to be expended often in the form of ATP or some other high energy intermediate. Among the conjugation reactions, glucuronide formation is exclusively microsomal. These syntheses are divided into groups according to the common species which is being used to derivatize the metabolite.

Let us for a moment consider the enzymes responsible for oxidation, the oxidases. Some are very specific as to the type of substrate which they will oxidize; others are relatively nonspecific, such as the mixed function oxidases; while some have a limited functional specificity such as monoamine oxidase, diamine oxidase and xanthine oxidase. Notice that the specific name for each enzyme will related in some way to its substrate. All oxidases need to have a cofactor to which electrons can be transferred. O_2 is a direct electron receiver for some reactions while NADPH and flavoprotein complexes known as the cytochrome Ps can be involved in a complex transfer of reducing and oxidizing power. Cytochrome P_{450} is a well-studied oxidizing system and is illustrated in Figure 9. (The number which is added as a subscript to the P family refers to the wavelength of maximum absorbance for the species.)

3. Nonsynthetic Reactions

a. Oxidation - Recall that the term oxidation refers to the loss of electrons which may be evidenced by the loss of hydrogen atoms or the addition of oxygen atoms. Oxidation cannot proceed without a substance to receive the lost electrons. The species receiving electrons is undergoing reduction. Converse to the case of oxidation, reduction may involve the gain of electrons or hydrogen atoms or the loss of oxygen atoms.

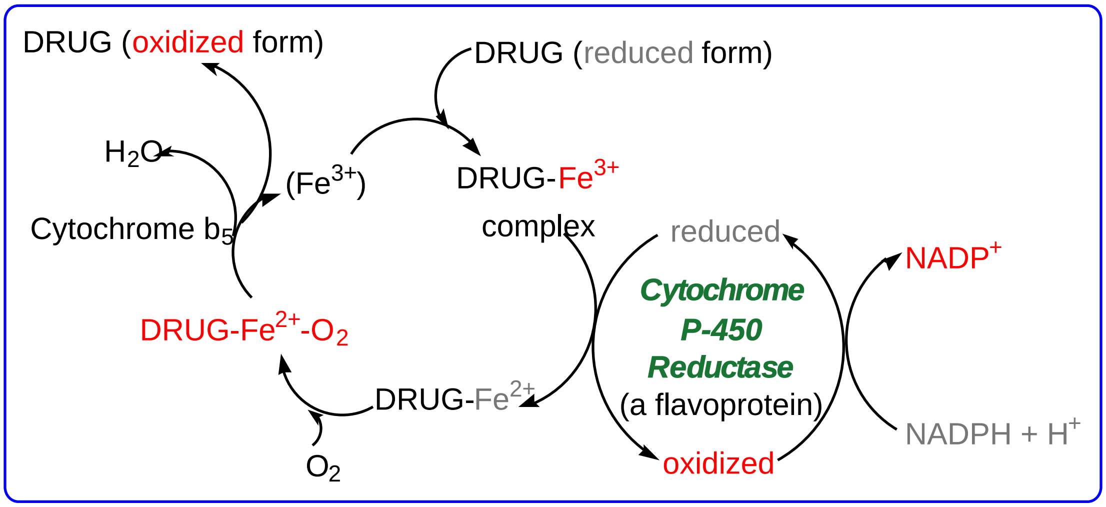
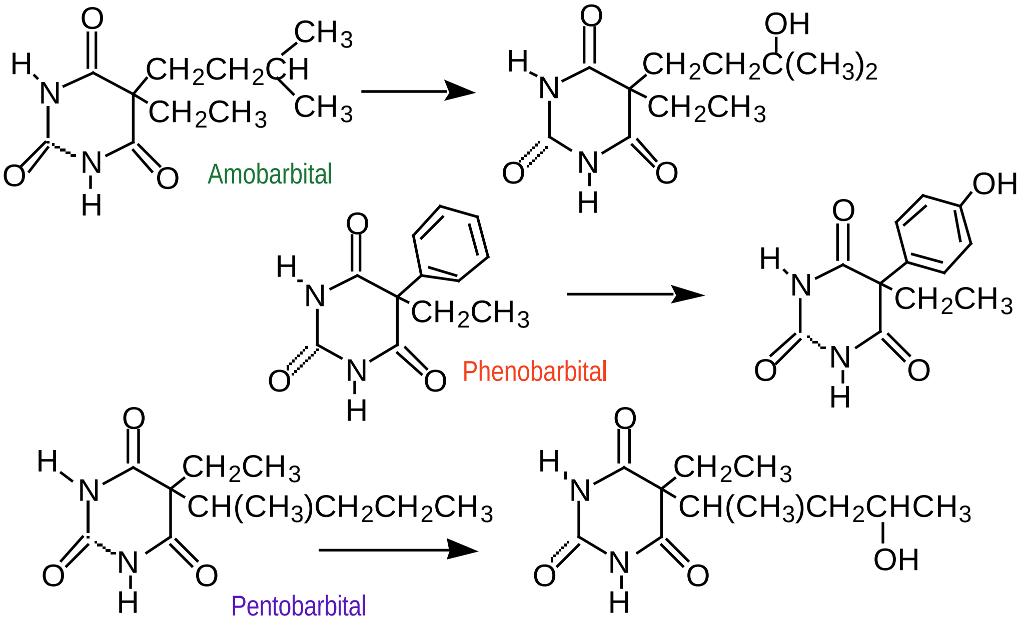


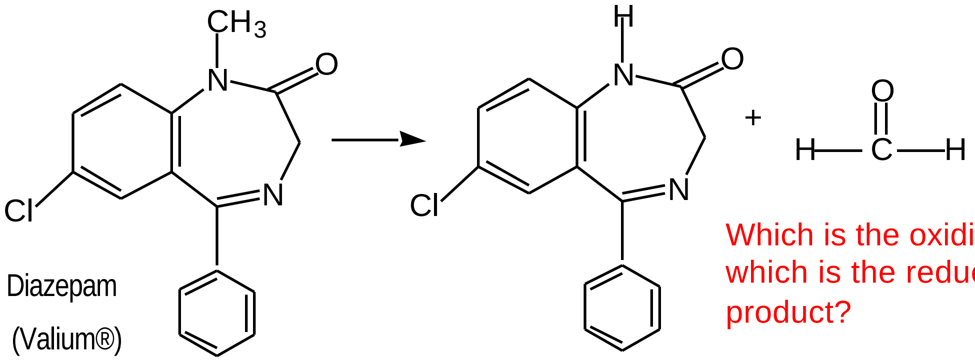
Figure 9
The Cytochrome P_{450} System

1. hydroxylations (aliphatic, aromatic, nitrogen)

The following three reactions involve the central nervous system depressants known as barbiturates.



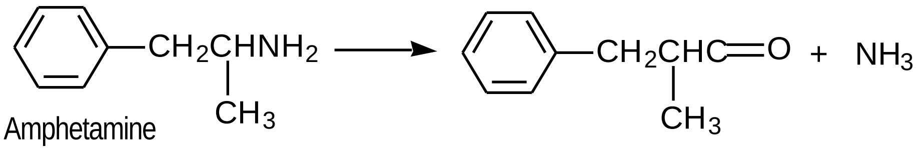
2. dealkylations (N-, S-, O-)



Diazepam
(Valium®)

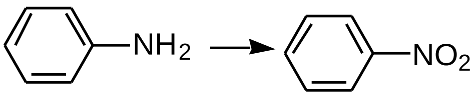
Which is the oxidized and which is the reduced product?

3. deamination



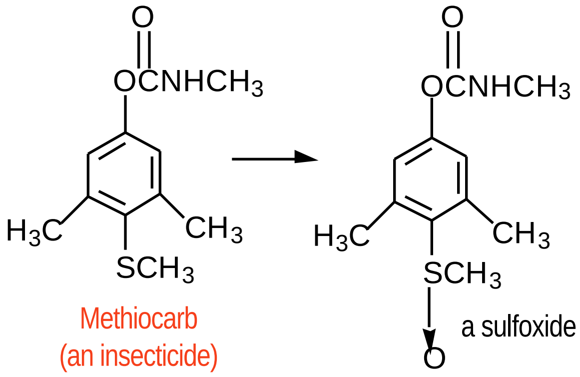
Amphetamine

4. N-, S-, and P- oxidation



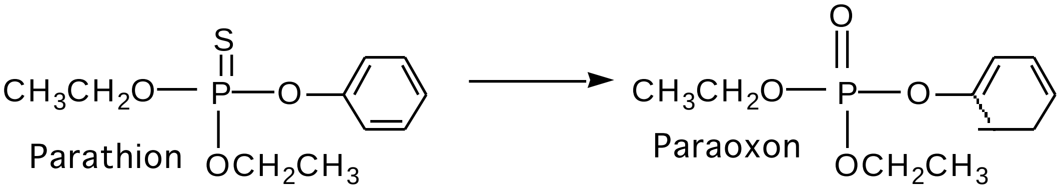
Aniline

Nitrobenzene



Methiocarb
(an insecticide)

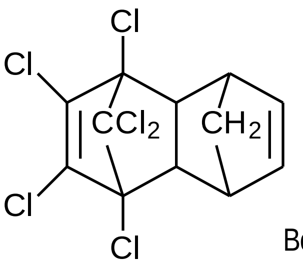
5. S-replacement



Parathion

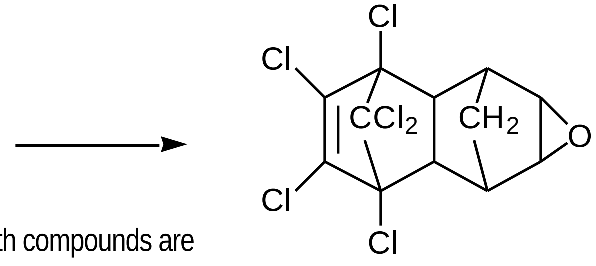
Paraoxon

6. Epoxidation



Aldrin

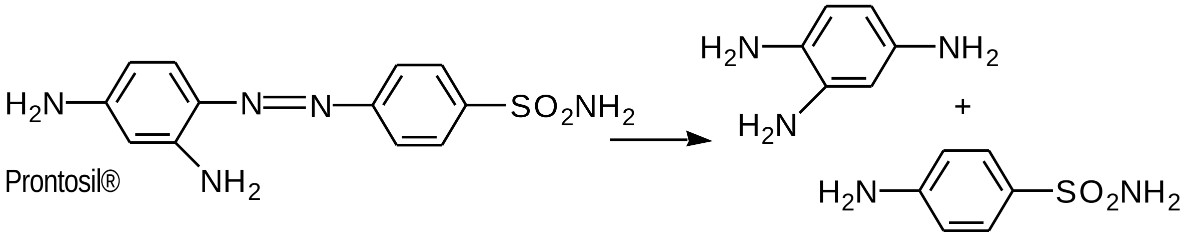
Both compounds are insecticides.



Dieldrin

b. Reduction (These reactions are catalyzed by reductases and they require cofactors for the reaction to occur such as $NADP^+$, FAD, etc.)

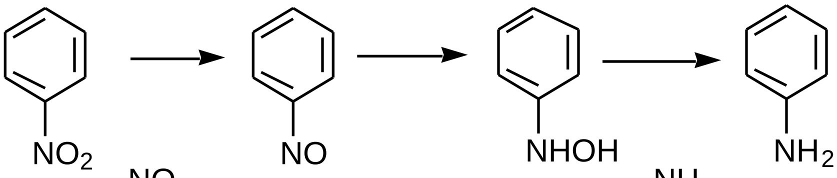
1. Azo reduction



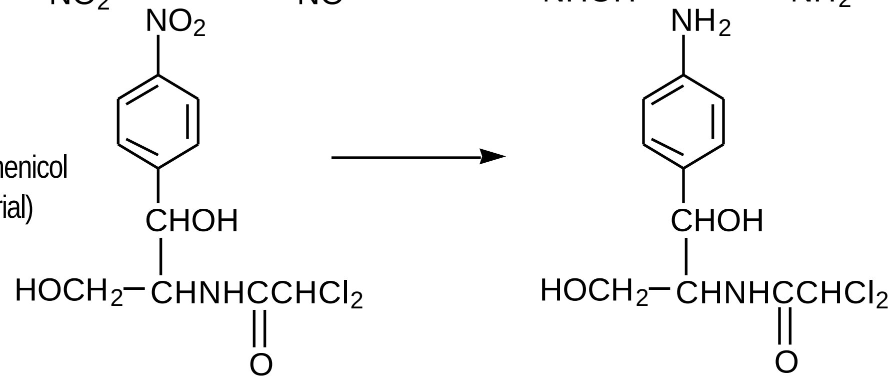
Prontosil®

sulfanilamide

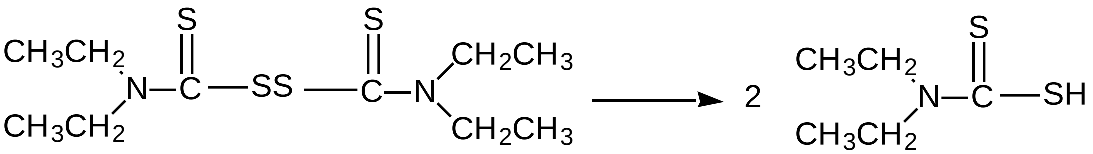
2. Nitro reduction



chloramphenicol
(antibacterial)

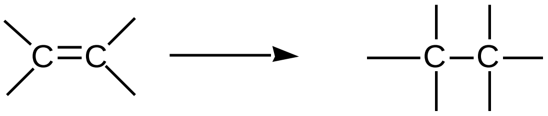


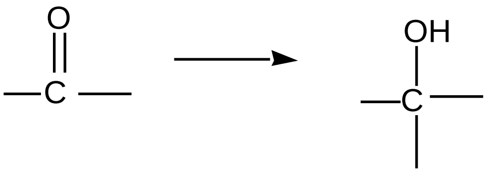
3. Disulfide reduction



Disulfiram (Anatabuse®)

4. Other types of reductions

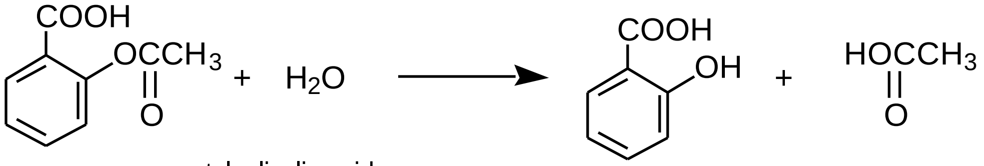




$As^{5+} \longrightarrow As^{3+}$

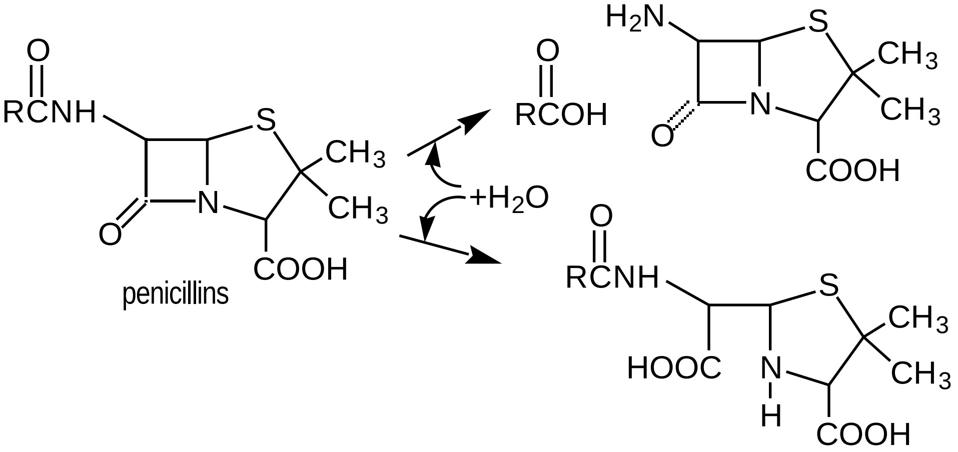
c. Hydrolysis (catalyzed by ***hydrolases***) -Substrates are ester, amide, glycoside and other water hydrolyzable bonds.

1. esters



acetylsalicylic acid

2. amides

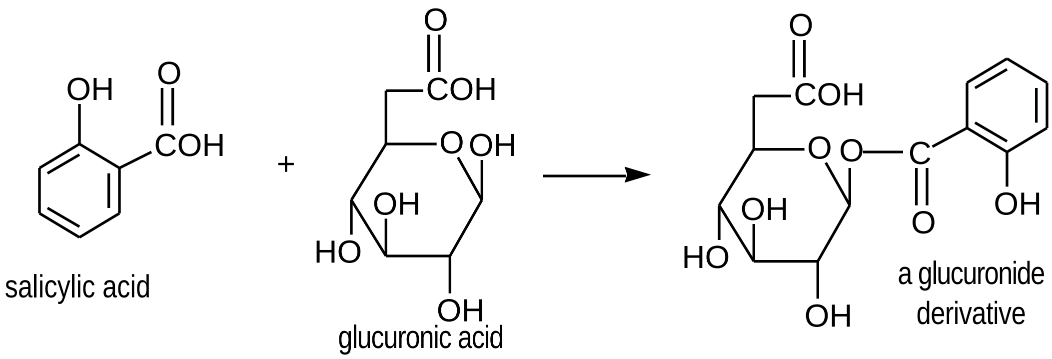


penicillins

4. **Synthetic Reactions**

a. Glucuronide Formation - Glucose can be used for more than an energy source. It can be oxidized into various products that can be used for detoxification processes and for coenzyme activity. Oxidation of the aldehyde group of glucose produces gluc<u>onic</u> acid while oxidation of the alcohol group on C-6 produces gluc<u>uronic</u> acid. Further oxidation gives ascorbic acid, or vitamin C. Most animals can

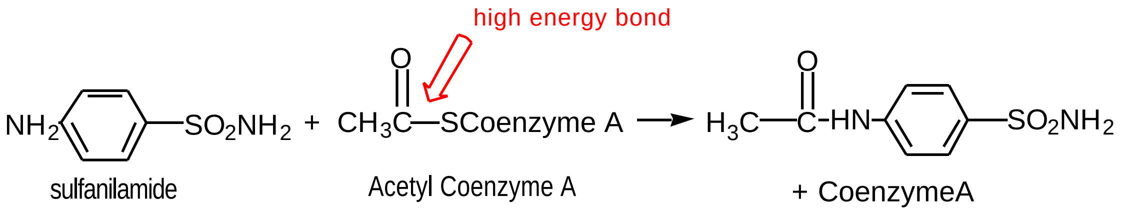
produce ascorbic acid. Guinea pigs, the Indian fruit bat, and higher primates, including humans, cannot make their own vitamin C (which is why it is a vitamin to us).



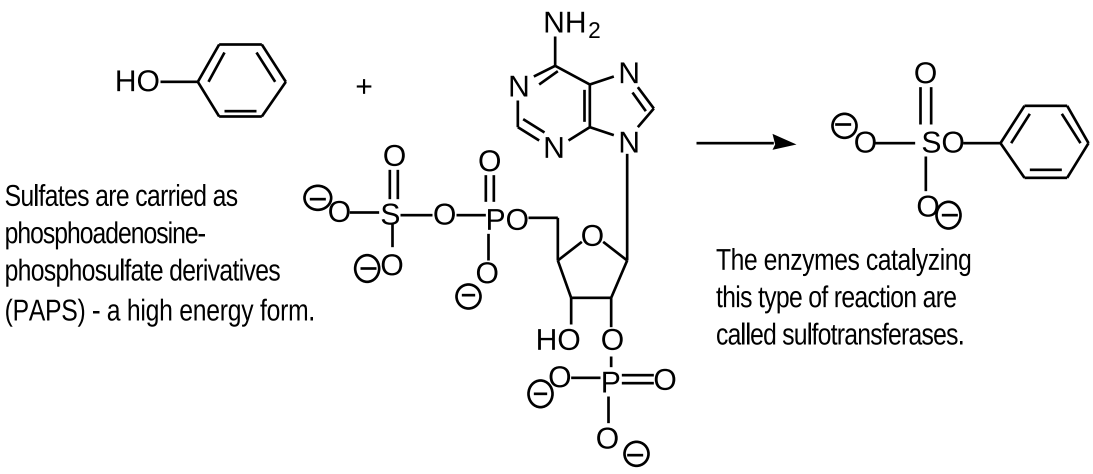
Since this is a synthetic reaction, that is, a bond-making reaction, energy is needed in the form of a nucleotide phosphate cofactor. Glucuronic acid is present as its high energy uridine diphosphate (UDP) derivative. The process of making this derivative requires the hydrolysis of a high-energy phosphate bond.

b. Esterification

1. acetylation

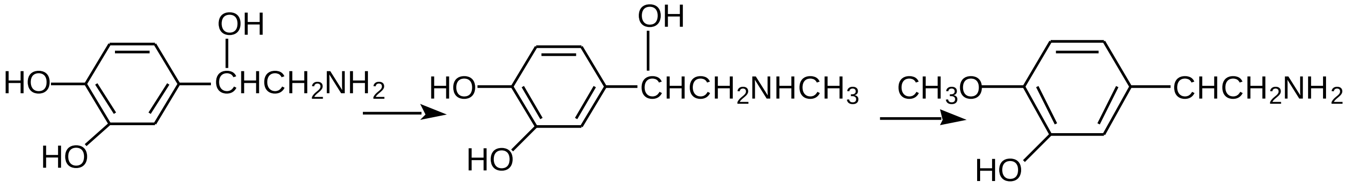


2. sulfate ester formation



Phosphate esters can also be formed but are not a common synthetic product in higher organisms.

c. Methylation (N-, O-, S-, and other elements)

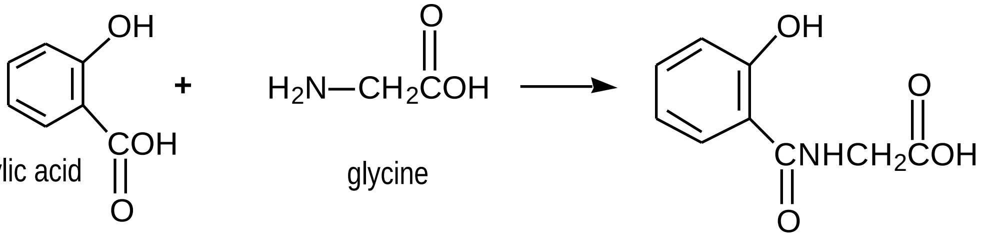


norepinephrine epinephrine metanephrine

$$Hg^{2+} \longrightarrow CH_3Hg^{+} \longrightarrow (CH_3)_2Hg$$

dimethylmercury

d. Amino Acid Conjugation



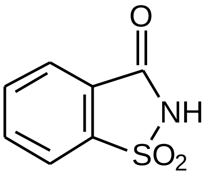
salicylic acid glycine

The amino acid glutamine can also be used as can glutathione (γ-glutamylcysteinylglycine) for conjugation. For example, the cancer chemotherapeutic agent methotrexate participates in polyglutamation similar to the vitamin folic acid. The use of other amino acids for this purpose is species-specific. Ornithine (similar in structure to lysine) is used in reptiles. Arginine and glutamine conjugates can be found in ticks. The sulfate amino acid analogue, taurine, conjugates with xenbiotics in fish. Alanine, serine, glycine, and glutamine are used in insects.

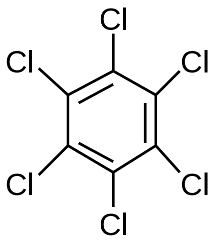
Some substances are resistant to biotransformation. Ther are various reasons for such resistance that we do not have the time to pursue in this course.

Some examples of resistant materials are

$CH_3CH_2OCH_2CH_3$ diethyl ether



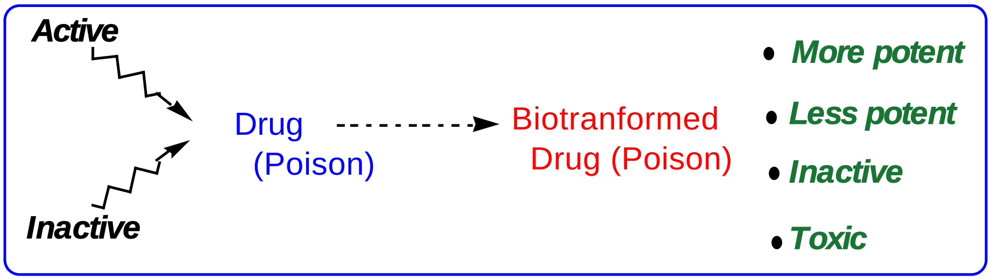
saccharin
artificial sweetener



hexachlorobenzene
fungicide

5. Results of Biotransformation

The enzymatic route which a drug or poison follows in its metabolism is very specific to the xenobiotic itself. Substances with the same type of structures need not go through the same pathway. And the "active" form of the drug or the "toxic" form of the poison may occur either at the beginning or during the course of transformation. Usually synthetic combinations are not active or toxic but many of their precursors are. The following diagram illustrates the possibilities of a biotransformation process.



Consider the following examples of changes which affect the activity of the material in question.

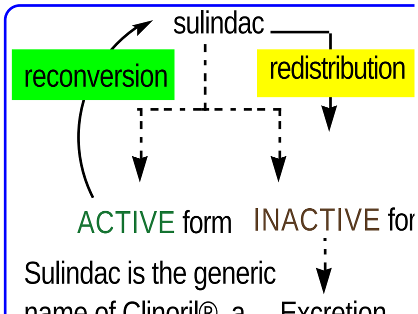
Acetylsalicylic Acid ------► Salicylic acid
ACTIVE analgesic → ACTIVE analgesic

Codeine ------► Morphine
ACTIVE narcotic analgesic → ACTIVE (more potent) narcotic analgesic

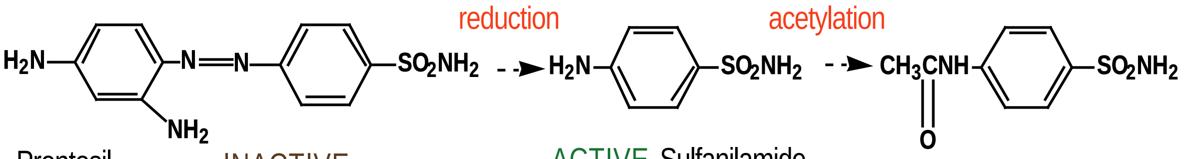
Malathion ------► Malaoxon
INACTIVE Insecticide → TOXIC (☠) Insecticide

Methanol ------► Formaldehyde ------► Formic Acid
ACTIVE CNS depressant → TOXIC (☠) → TOXIC (☠)

Digitoxin ------► Digoxin
ACTIVE cardiac stimulant → ACTIVE cardiac stimulant



Sulindac is the generic name of Clinoril®, a NonSteroidal AntiInflammatory Drug (NSAID).



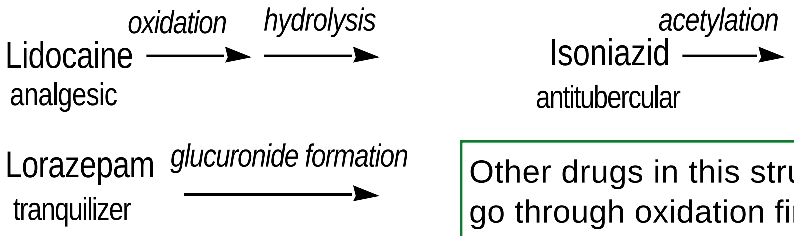
Prontosil INACTIVE → ACTIVE Sulfanilamide → INACTIVE Acetylsulfamilamide

6. **Factors Affecting the Rate of Biotransformation**

The individual reactions of drug metabolism will proceed at different rates depending upon the substrate (drug or metabolite), the enzyme catalyzing the particular reaction, and any other interfering agents. If the overall rate of biotransformation is slow, then the effects being observed are those primarily of the parent compound itself and perhaps, to a limited extent, to one or more of the initial metabolites. When the overall rate is fast, the effects must be due to the metabolite(s).

a. Pathways - The usual pathway of metabolism for a xenobiotic is from nonsynthetic to synthetic reactions. However, this is a generality, it is not a rule. The route is drug-specific and may vary within a particular structural class of compounds. Also we can find that the nonsynthetic metabolites are more likely to have some activity or toxicity while the synthetic conjugates are most often devoid of activity or toxicity.

The following are some examples of the variation of biotransforming pathways.



Other drugs in this structural/pharmacological class go through oxidation first. This is important when prescribing combinations of drugs which might compete for the enzymes of biotransformation.

Different species have developed different pathways and this can have a significant impact on their use. Consider the metabolism of the insecticide malathion.

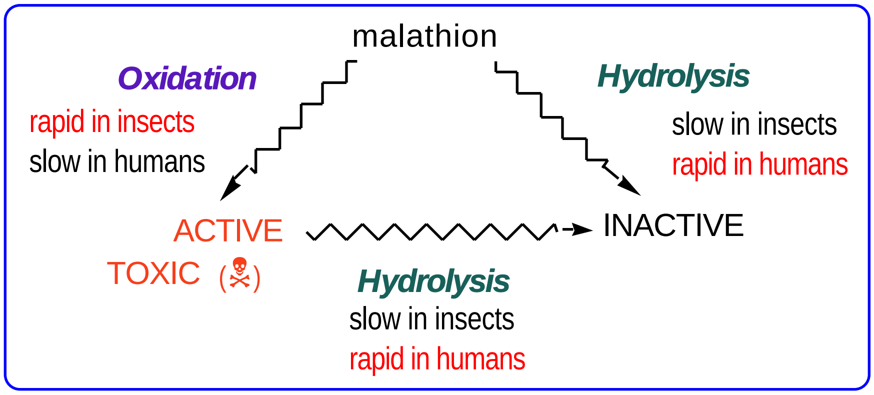


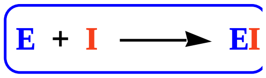
Figure 10
Comparative Pathways of Biotransformation

A rapid oxidation process in insects converts the relatively inocuous malathion to the toxic malaoxon. Detoxification by hydrolysis proceeds at much slower rate. Therefore the toxic species buildups up in the the insect and eventually kills it. However, in humans the process is reversed. The detoxifying hydrolysis reaction is faster that the toxifying oxidation. The result is an insecticide which can be used by humans with relative safety.

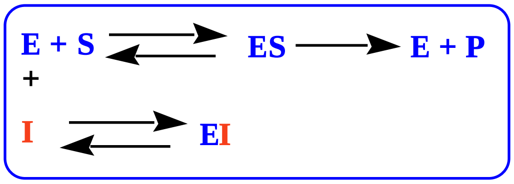
Since biotransformation involves enzymes, there are many factors which can affect enzyme performance in a positive or negative way.

b. Enzyme Inhibition - Recall from your study of biochemistry that enzymes may be inhibited either reversibly or irreversibly. Many drugs and poisons have their main effect or significant side effects by acting as enzyme inhibitors. This can be especially important when considering the biotransformation of those drugs or of some other endogenous or exogenous molecules. Let us consider the various types of inhibition and some important examples.

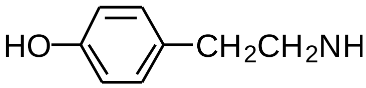
1. Irreversible inhibition - This type of inhibition involves the stable combination of an enzyme with some species so that the only way to have enzyme activity is to produce more enzyme. Specific examples of irreversible inhibition will be covered in Part II of the course in the section on the nervous system. Organophosphate and carbamate insecticides are the prime examples of such inhibitors when they combine with the enzyme acetylcholinesterase.



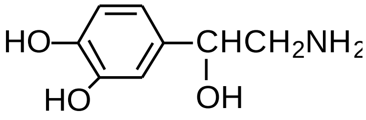
2. Reversible competitive inhibition is also common. This impairment of an enzyme is due to the fact that the inhibitor (drug or poison or even natural material) looks like the normal substrate for the enzyme. Using the abbreviations E, S and I for enzyme, substrate and inhibitor, respectively, we can discuss several examples of competitive inhibition.



Tyramine is a natural metabolite of the amino acids phenylalanine and tyrosine. Its structure is very similar to that of epinephrine and norepinephrine which stimulate the nervous system resulting in increased heart rate and blood pressure. All three of these molecules are metabolized by monoamine oxidases. As you will learn later on in the course, people suffering from depression are believed to suffer from a deficiency of norepinephrine and/or dopamine, a closely related compound. Therefore, one of the types of medications given for depression is a monoamine oxidase inhibitor such as isocarboxazid (Marplan®). The interesting



tyramine



norepinephrine

aspect of this prescription is that patients using monoamine oxidase inhibitors (MAO inhibitors) must be careful to avoid certain types of decongestants which contain norepinephrine-like molecules. Excessive quantities of these compounds could have a fatal effect on blood pressure. In addition, such patients must also avoid certain types of foods which naturally contain tyramine: chicken livers, red wine, pickled herring and Camembert, Stilton and Roquefort cheeses.

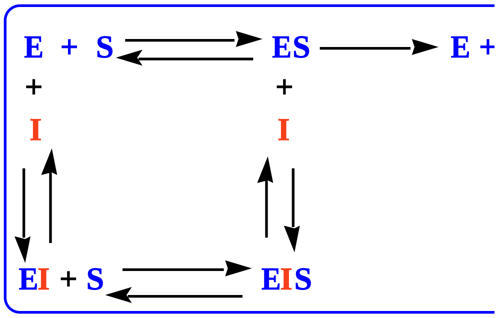
Two substrates vying for the same enzyme can be used to advantage as occurs in the use of ethanol as an antidote for ethylene glycol (antifreeze) or methanol (wood alcohol) poisoning. All of these molecules are oxidized by liver alcohol dehydrogenase (ADH). Eventually the ethylene glycol will be oxidized completely to oxalic acid and methanol to formic acid, both of which are toxic, while ethanol is converted to ethanoic or acetic acid which is a natural metabolite. The ethanol is a "better" substrate for the ADH and is preferentially oxidized. Consequently the ethylene glycol and methanol will not be converted to their toxic forms and, being water soluble, they will be excreted in the urine.

$HOCH_2CH_2OH$
ethylene glycol

CH_3OH
methanol

CH_3CH_2OH
ethanol

3. Reversible noncompetitive inhibition - The types of substances which can fit this inhibitor category need not look like a normal substrate. That's because they can bind to the enzyme whether the normal substrate is already bound or not.



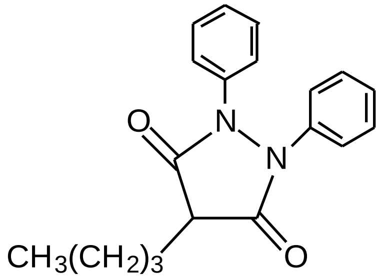
Examples of noncompetitive inhibitors are the heavy metals which act as poisons such as lead, mercury and silver ions. Mercury is attracted to -SH(thiol) groups in the active sites of enzymes which use thiols in their catalytic mechanism. It should be noted that heavy metal ion poisoning can be overcome using chelating agents which tie up the ions in a stable complex, pulling them away from the enzyme.

We have already discussed a therapeutic application of inhibition in the example of ethanol being used as an antidote to ethylene glycol or methanol poisoning. There are many other such cases which could also be cited. Antabuse®, disulfiram, prevents the metabolism of ethanol. As a result a person under treatment with Antabuse will become violently ill if s/he consumes ethanol. Barbiturates are rapidly metabolized especially if a person has been on a prescription for some time. Administering the antibacterial chloramphenicol will inhibit the breakdown of barbiturates and in so doing prolong their sedative action.

c. Enzyme Induction - Many xenobiotics, not just drugs but also natural foods and pollutants, have the ability to enhance the rate of biotransformation. Th is what is know as **induction**. It can even be seen in electron micrographs (

induction of biotransformatio

the endoplasmic reticulum when such chemicals are used. ER content overall is larger both on the smooth and rough sides. Recall that the rough endoplasmic reticulum is responsible for the biosynthesis of protein. The substances which cause induction can therefore affect the rate of their own metabolism or the rate of biotransformation of some other substance or both. The following are some examples of substances which can cause induction and the metabolisms which they induce.



phenylbutazone

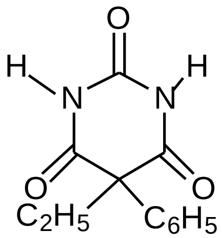
Phenylbutazone is an antiinflammatory drug which not only induces its own biotransformation but will also enhance that for aminopyrine (analgesic) and cortisol (antiinflammatory).

Ethanol, the well-known CNS depressant, induces itself and other types of depressants such as barbiturates. This means that over a period of time, more ethanol or barbiturate must be consumed in order to achieve the state which was achievable at a lower dose early on in its use. This offers a partial explanation of the concept of tolerance to a drug's effects. **Tolerance** is the need to take an ever larger dose of a drug in order to experience comparable effects. Because of the induction of other sedatives though it becomes very difficult to sedate an alcoholic using those drugs. Please note that tolerance does not mean that it becomes harder to overdose on either ethanol or barbiturates. The lethal dose, which will be discussed shortly, remains the same. Therefore the tolerant individual, if s/he continues increasing her/his dose, will come closer to killing her/himself. Another item which should be noted with regard to this combination. The induction effect just mentioned refers to taking the ethanol and barbiturates separately and not in combination. If the two are taken in combination they will compete with each other for biotransformation and the concentration of free, unchanged drugs in the blood will be higher than if each were taken alone, resulting in a greater than anticipated cumulative effect. This is known as **synergism**.

tolerance

synergism

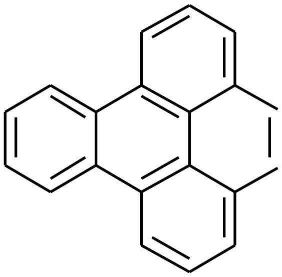
Phenobarbital is an inducer not only of itself but also of the hormone testosterone, the anticoagulants dicoumerol and warfarin, the cardiac stimulant digitoxin, and a host of others. Now consider the following situation. Phenobarbital is often given in combination with dicoumerol in order to alleviate the anxiety which accompanies a blood clotting disorder. The dose of the anticoagulant must be increased carefully to compensate for the inductive effects of the barbiturate. It is easy to see how the patient, feeling well and less apprehensive, might decide to slack off on her/his medication, that is, on the barbiturate portion of it. What will happen? Induction will decrease and the amount of active anticoagulant in the patient's bloodstream will increase. A hemmorhage can result. Therefore there is a need to constantly monitor the clotting, or prothombin, time of someone under such treatment. Patient compliance is an important part of any drug therapy.



phenobarbital

Benzo(a)pyrene is a product of the burning of cigarettes and meat. The structure shown is a precarcinogenic form, that is, it is converted in the

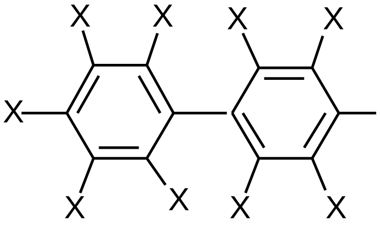
body by the processes we were just discussing to a cancer-formed compound. And it induces itself as well as other polycyclic aromatics formed in the combustion process.



benzo(a)pyrene

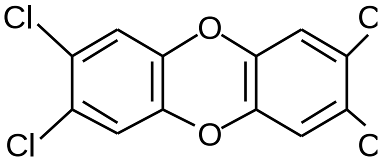
Polyhalogenated hydrocarbons such as PCBs and PBBs cause such extensive induction in theliver and kidneys that the liver will dramatically increase in size and weight. TCDD, a dioxin, also alters some drug biotransformations as does DDT.

And to add some fun to this process, the indoles found in cruciferous vegetables such as broccoli, cabbage, and brussel sprouts are also inducers. In fact, Dr. Bruce Ames, UC Berkeley, a prominent voice in the great cancer debate, points out that there are positive and negative aspects to induction. That caused but natural foods such as broccoli may have a positive effect in ridding the body of cancer-causing xenobiotics.



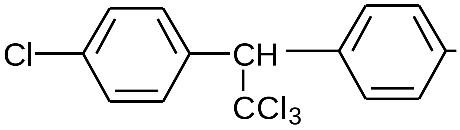
polychlorinated (X=Cl) and
polybrominated (X=Br)
biphenyls (PCBs and PBB

<u>c. Individual Variations</u> - There are many variations in the rate of biotransformation which can exist depending upon the type of organism, its own particular genetic makeup, the stage of development, sex, weight, diet, and so on. In humans there may be up to a six-fold variation in "normal" drug metabolism. The doses prescribed for humans are based upon an arbitrary standard often a 20 year old, 70 kg male. (Well, who else needs the money to get through school?). Therefore it is imperative that the patient report whether the prescribed dose is sufficientm too little or too much by observing side effects. The use of such a standard is obviously the source of contention. With the current AIDS crisis as well as an aging population and heightened sensitivity to issues of gender and race, we will see the testing and validatin of drugs on a wider population base in the near future.



tetrachlorodibenzo-p-dioxin
TCDD

Since everyone is different genetically it is obvious that the processes of absorption, distribution and biotransformation may be extremely individualized. Add to this that we still have to consider excretion, the concept of individual drug receptors in cells and the immune system and you can see that having any type of standard for the entire picture is a fantasy. Also consider that trials in humans are the last phase of any drug study. Animals, with all of their variations, are the first in line.



dichlorodiphenyltrichloroethane
DDT

An interesting example of racial differences in drug conversion is seen in the metabolism of the antitubercular, isoniazid. It is inactivated by an acetylation reaction. Slow acetylation leads to toxicity (lupus, drowsiness, nausea, cyanosis). Free isoniazid also inhibits the action of phenytoin (an anticonvulsive) and results in phenytoin toxicity. Normal acetylation has a half-life of 45-80 minutes while a "slow acetylator" shows a 140-200 min half-life. The U.S. population shows a 50/50 distribution of "slow" versus "fast" acetylators. 44-55% of American Causasians and blacks are "slow",

while 60% of Europeans are "slow". In contrast, only 5% of Eskimos are "slow acetylators".

A couple of further examples can help to substantiate this point. Lately it has been found that it takes ten times as much haloperidol to relieve schizophrenic symptoms in Caucasians as it does in the inhabitants of Taiwan. And when using propanolol for the control of blood pressure, men of Chinese descent metabolize it more efficiently than Caucasians and could use lower doses for comparable effects.

In the fetus and neonate the systems of biotransformation are poorly developed. Specifically oxidation and conjugation reactions are negligible. The blood-brain barrier is poor and the excretory system is immature. This is nowhere more evident that in the number of babies who are born with jaundice caused by the incomplete breakdown of heme. Heme normally undergoes conversion to bilirubin and then conjugates with glucuronic acid for excretion. This last step is deficient in many babies and the bilirubin builds up causing a yellow color in the child's skin (hyperbilirubinemia). The baby can be treated by exposure to UV light which alters the conformation of the bilirubin and allows for its excretion. The inability for a fetus or newborn to biotransform drugs must be taken into account especially when treating a mother since we have to keep in mind the permeability of the placental membrane and the fact that most drugs can be passed in breast milk.

The metabolism of a growing child is not like that of the standard adult. Some systems are faster, some are slower. It is very hard to determine the exact state of children because it is unethical to experiment with them. The prevailing philosophy is to scale down an adult dose according to the weight ratio with an adult.

By the year 2000 12.5% of the population of the United States will be "senior citizens". This brings another set of criteria to be considered in prescribing medication. As we age our ability to absorb drugs and excrete them becomes slowed. A decline in organ integrity causes changes in biotransformation especially in the nonsynthetic portion. For example, the half life of the tranquilizer diazepam has been found to be related to a patient's age in years. Therefore its is recommended that the initial dose of this drug be 30-50% that for a younger person and that the dose be adjusted when the effects and side effects are considered. The senior citizen is also more likely to be on a complex regimen of medications, many of which can interact with each other. Analgesics are a common frequently prescribed drug for the older person. Darvon®, propoxyphene, has reached sales of 30 million prescriptions per year for this purpose. However, the drug is also responsible for 1 to 2 thousand fatalities per year because it impedes the metabolism of other drugs. For example, a recent news article told of an 89 year old man who had been taking Darvon for his arthritis and doxepin for depression. His blood level of doxepin was twice that expected.

With all of these things to consider it's a wonder that we have any pharmaceutical industry at all.

E. Excretion

Once a drug is biotransformed, it has to be eliminated from the body. Although the main exit is through the kidneys, materials can also be excreted in the feces and through sweat, tears and milk. Some molecules are not easily converted from lipophilic to hydrophilic and so are reabsorbed, eventually finding their way into the bile and large intestine. In fact, there are transport systems which will actually secrete some drugs into the bile.

1. The Function of the Kidneys

We are each born with two functioning kidneys, 6 inch long, bean-shaped organs located in the abdomen behind the other organs. The three general parts of each kidney are the pelvis, where the blood supply enters and leaves the organ, and the medulla and the cortex, where the process of filtration takes place. The flow of blood becomes diffused as it enters the kidney and so flow is lowest in the pelvic region and the highest in the cortex. The medulla and cortex contain the working filtration apparatus of this organ - the nephron. In fact each kidney contains about one million nephrons. This means about 75 miles of tubing which filter out water and undesirable materials from the blood as well as allow for the reabsorption of necessary molecules. About one-fifth of the entire blood supply will enter the nephrons per minute (~1200 mL/min). This means that it takes about 4-5 minutes to filter the total blood volume. Amazing, isn't it!

Besides the excretion of waste material, the kidneys serve many other vital roles. Since water and salts can be excreted or reabsorbed, the kidneys will help to regulate metabolite and salt concentrations within the body and maintain the total volume of fluids. Any rapid loss in fluid volume results in lowered blood pressure (shock). Retention of water can cause **edema** while excess secretion of water results in **dehydration**. In addition, this organ regulates acid-base balance (pH of urine can be between 4.5 and 8) and produces hormones necessary for cell production (erythropoietin), calcium and phosphorous regulation(the active form of vitamin D), blood pressure (renin-angiotensin-aldosterone), as well as synthesizes prostaglandins and kinins which serve many roles and degrades endogeneous biomolecules such as insulin and glucagon. Figure 11 is a diagram of a kidney.

edema

The anatomy of a nephron is a key to understanding how the kidney functions. Refer to Figure 12 which schematically illustrates each part. The blood enters the kidney through an artery into a knot of vessels known as the glomerulus. The glomerulus is surrounded by a loose

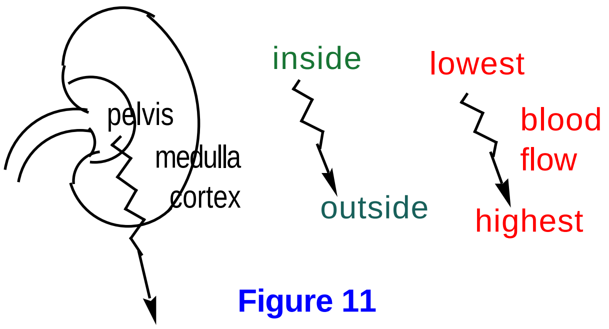


Figure 11
The Kidney

aggregation of cells known as Bowman's capsule. The capsule contains

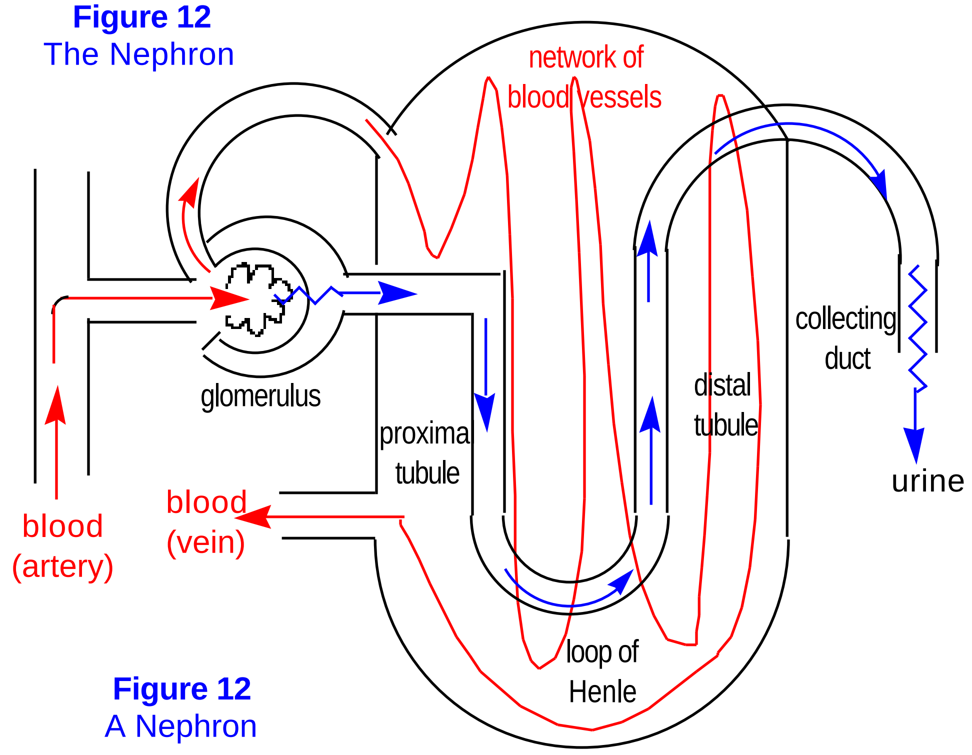


Figure 12
A Nephron

pores up to 40 Angstroms (10^{-8} cm) in diameter. This allows anything up to a molecular weight of about 15,000 daltons to be forced by the intrinsic pressure of the blood through the capsule and into the kidney nephron tubule. The residual blood supply is routed through a diffuse network which surrounds the nephrons in the medulla and cortical areas of the kidney. The crude filtrate enters the proximal tubule , then passes through the loop of Henle, the distal tubule and into the collecting duct on its way to the ureter. The entire path is more complex than it appears in Figure 12, but the idea is there.

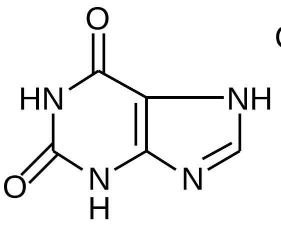
The processes of selective reabsorption of nutrients and xenobiotics goes on within the complex tubule system. 98-99% of filtered materials (salts, water, sugars, amino acids) are eventually reabsorbed by passive or active transport. Biomolecules such as glucose and amino acids are entirely reabsorbed if their concentrations are within the normal range in the blood. However, should the concentrations be higher than normal, those molecules might not be completely reabsorbed because they have exceeded the ability of the nephron transport systems to accommodate them. This is referred to as exceeding the renal threshold. Urine is therefore a convenient body fluid to assay for the initial assessment of metabolic or excretory system malfunctions.

Different portions of the nephron have selective water permeability. Sodium and potassium ions are reabsorbed in the proximal tubule and distal tubules and water will pass at these sites to maintain osmotic

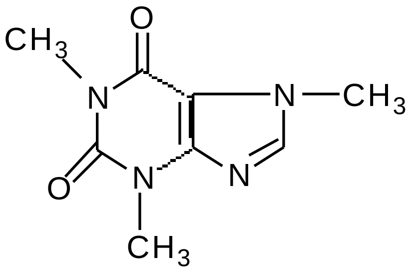
pressure. In addition chloride ion will be reabsorbed to counterbalance the positive ions. In the loop of Henle Na^+ and Cl^- ions will be reabsorbed without water. Water absorption is controlled in in the distal tubule and collecting duct by the action of the hormone ADH, antidiuretic hormone or vasopressin. In the absence of ADH, produced in the pituitary gland, the tubule is impermeable to water and the urine will be hypotonic (watered-down). A normal state implies some ADH activity which means that the urine will normally be hypertonic because water is being reabsorbed into the blood.

Antidiuretic hormone is an integral part of the renin-angiotensin system which functions in the regulation of salt concentrations and fluid volume, thereby helping to control blood pressure in the body. A deficiency of ADH causes a condition known as diabetes insipidus. Ethanol and caffeine inhibit the activity of ADH by affecting the functioning of a second messenger system resulting in diuresis. (It's not just the volume of beer or coffee which causes excessive urination, it's also the chemicals present.)

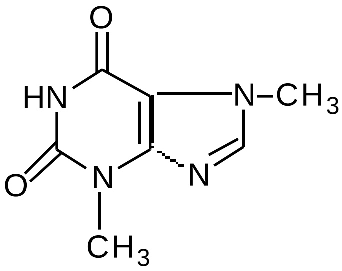
ASIDE# 2 - METHYL XANTHINES



xanthine



caffeine
coffee bean, tea leaves,
cocoa seed, kola nut

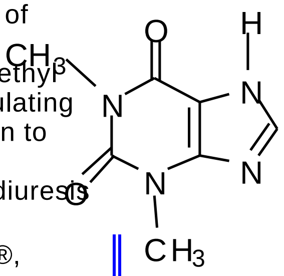


theobromine
cacao bean, kola nuts, tea

Theoretical explanations for the stimulatory activity of the methyl xanthines:

- These compounds inhibit phosphodiesterase resulting in the prolonged activation of metabolic enzymes.
- Adenylate cyclase activity is modulated by the binding of free adenosine. The methyl xanthines are antagonists to adenosine but their bindings do not produce its modulating (inhibitory, attenuating) response. Therefore the end result is stimulatory in relation to cAMP activity.

Effects: relaxation of bronchi, increase in respiration, stimulation of cardiac muscle, diuresis
Methyl xanthines are used in the treatment of asthma.



This is theophylline or aminophylline (theophylline plus ethylene diamine) Slo-bid®, Bronkodyl®, Theo-Dur®, etc. available in capsules, caplets, liquids, elixirs, syrups, suspensions.
(an **elixir** is a sweetened, alcohol extract or solution of the material)

The membranes of the tubules contain enzyme and active transport systems. One such important enzyme system is carbonic anhydrase, which you might remember as being necessary for establishing the blood-buffer (bicarbonate) system. It performs the same buffering function here allowing

bicarbonate to be reabsorbed in the proximal and distal tubules and participating in an exchange route for sodium ion. See Figure 13.

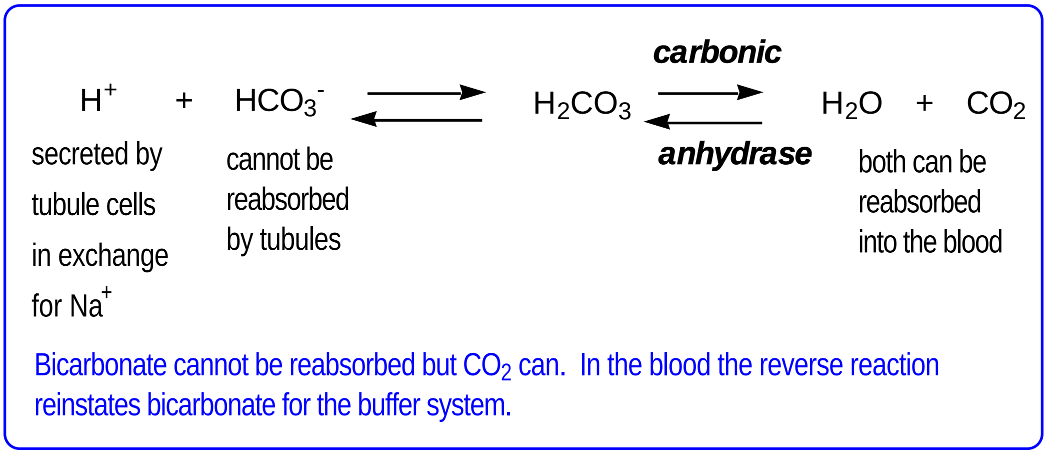


Figure 13
Kidney Carbonic Anhydrase System

If alkali is being excreted, H^+ secreted by the tubule cells neutralizes it and bicarbonate is excreted. The pH of the urine will rise.

Two tubular secretory systems exist for the active transport of organic acids and organic bases. Many xenobiotics can find their way back into the blood via these routes.

2. Excretion of Drugs and Poisons

The main factor in determining whether or not a drug or poison will be excreted via the urinary tract is its lipophilicity. If the material is lipophilic it can be reabsorbed by passive diffusion. The glomerular filtration rate, individual tubule secretory systems, and tubule exchange systems will help to establish excretion rates. Because a molecule's lipophilicity can be pH-dependent the acidity of the urine is extremely important.

Aspirin was once the leading cause of poisoning in young children before the advent of the child-proof cap on OTC bottles. Remember that the pK_a of acetylsalicyclic acid (aspirin) is about 3.5. At the normal minimim pH of the urine, 7.4, less than one percent of the acid will be in its protonated, uncharged form and can be reabsorbed by passive diffusion. If the pH of the urine drops because of large amounts of H^+ present as in aspirin poisoning, than a larger amount (up to 10%) of the acid will be in the lipophilic form and be reabsorbed. As that 10% is reabsorbed the rest of the material will reestablish the ionization equilibrium so that another 10% will be in a lipophilic state and be subsequently reabsorbed and so on. This exponential rate of reabsorption can result in aspirin toxicity. (See Figure 14)

Phenobarbital poisoning is exacerbated by its ionized forms also. Its pK_a is about 7.2. At a urine pH of 7.4 there will exist about a 50:50 mixture of lipophilic and lipophobic species. Once again the uncharged form will be reabsorbed and the remaining barbiturate molecules will redistribute themselves according to the equilibrium eventually leading to the reabsorption of the virtually all of the phenobarbital.

In both the cases presented alleviation of the toxic situation could be accomplished if the urine was made more alkaline in order to drive the ionization equilibria to the conjugate base forms which are lipophobic and therefore excretable in the urine. Bicarbonate can be administered in order to raise the pH and help in the elimination of the drugs.

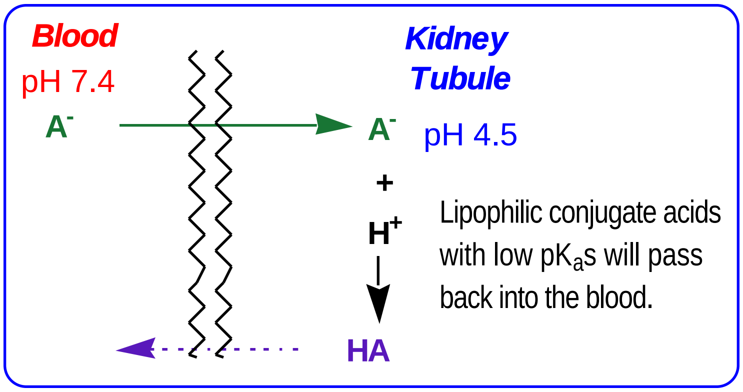
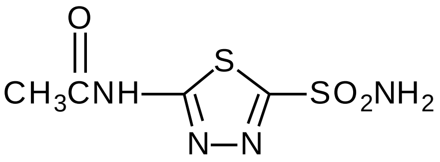


Figure 14
Reabsorption of Species from the Kidney Tubules

3. Diuretic Drugs

The control of water content has an effect on the blood pressure. Therefore drugs which can affect the balance of water or ions within the nephron can have a clinical use in the treatment of hypertension (high blood pressure). These pharmaceuticals are known as diuretics because their use results in an increased excretion of water. Since they all have the same general effect, diuresis, they represent a **pharmacological class** of compounds. Some may be related to each other on a structural level and be further associated as members of a **structural class** as well.

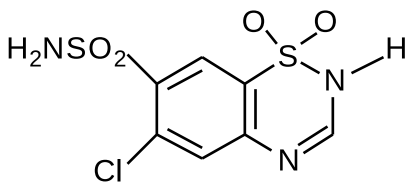
Sulfonamides, such as acetazolamide, although not used today for the treatment of hypertension, represent drugs which affect a specific enzyme system in the nephron. Sulfonamides are **carbonic anhydrase inhibitors**. By inhibiting this enzyme, bicarbonate cannot be reabsorbed and hydrogen ions will not be exchanged for sodium ions. The increased excretion of salt in the urine will draw water along with it and so cause diuresis and a



acetazolamide

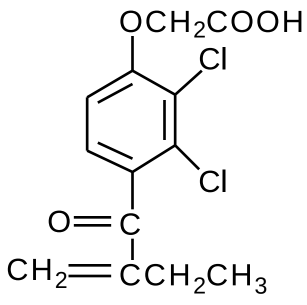
lowering of the fluid volume of the body which in turn lowers blood pressure. A undesirable but unavoidable side effect of these drugs involves an increase in blood $[H^+]$ and metabolic acidosis.

There are several other diuretics which fall into various structural categories.



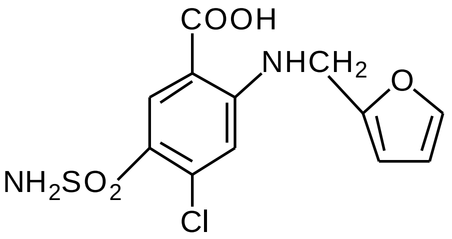
chlorothiazide
(Alurene®, Diuril®, Urinex®, Saluric®, among others)

The **thiazides** inhibit chloride ion reabsorption. Positive ions such as sodium and potassium also be exreted as counterbalancing ions. The sodium imbalance will also affect the bicarbonate ion excretion. All thiazides have similar potency at the maximum dosage.



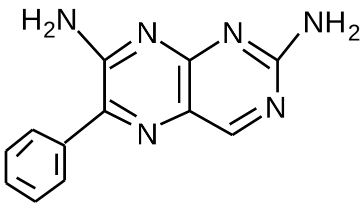
ethacrynic acid (Edecrin®)

Ethacrynic acid acts on the ascending portion of the loop of Henle as well as on the proximal and distal tubules to inhibit reabsorption of sodium ions. It is a much more potent agent than some of the other agents in this class and is called a "high ceiling" diuretic for that reason. Because of its activity in the loop of Henle it is also referred to as a "loop" diuretic.



furosemide (Lasix®)

Furosemide has a general effect of the reabsorption of sodium and chloride throughout the tubule system especially in the loop of Henle and is also a high-ceiling diuretic because of its potent, prompt onset of action (5 minutes after i.v. administration, within 1 hour when taken orally). Its mode of action is to inhibit the $Na^+/K^+/2\ Cl^-$ transport mechanism. This drug has been involved in sports drug scandals especially when it is used with racehorses which bleed from their lungs during events in order to lower their blood pressure during a race. Some states prohibit the use of any drugs including furosemide for this condition while other states allow it.



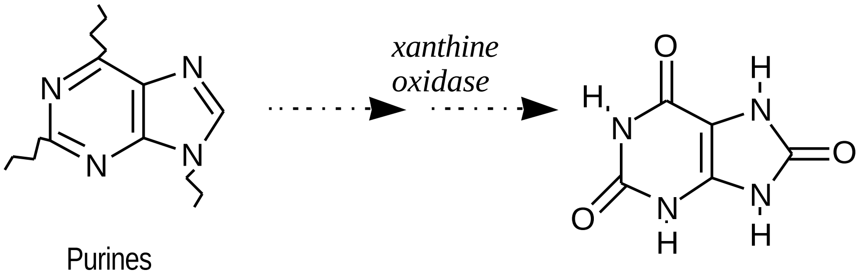
triamterene (Jatropur®, Dytac®)

Triamterene is known as a potassium-sparing diuretic. It interferes with the Na^+-H^+ exchange and can be used in combination with thiazides in order to help alleviate the electrolyte deficiency which can occur with diuretic treatment.

Diuretics in general reduce the renal clearance of Li^+ and can lead to lithium toxicity for those being trreated for manic-depression with lithium salts.

4. Gout - A Disorder Affecting Uric Acid Excretion

Nucleic acid metabolism results in the prodution of the burine base derivatives adenosine and guanosine. These bases can be reused or oxidized to uric acid for elimination from the body. Uric acid is secreted into the urine by the kidney tubules by an active transport system and is reabsorbed to a great extent. The total body uric acid content is about 1.1 grams, one-sixth of which is in the blood, the rest residing in tissues. Uric acid has two ionizable hydrogens. Its first pK_a is 5.75 so at physiological pH you would expect to find mainly its monobasic salt form. The acid and its salts have a very low solubility in water and should the uric acid content of the tissues increase or should the pH drop so as to convert the sodium urate salt to the less soluble acid form, uric acid crystals will form in the joints and kidney tubules (renal stones). The immune system will respond to this imbalance causing inflammation, swelling, and pain. A joint commonly affected is that in the big toe.



Purines

Uric acid

The imbalance in uric acid can have several causes - increased uric acid production by increased purine synthesis or breakdown, decreased renal excretion of uric acid, or decreased reuse of purines. Note that enzymes or transport proteins are involved in all of these possibilites. It is understandable then that the predisposition to this problem, known as gout, could be inheritable in that DNA codes for protein. In fact, the familial incidence of gout is 75-80% and usually occurs in males over thirty years old. Its occurence in women is postmenopausal and extremely rare in children and adolescents. Some great people and families have been the unlucky victims of gout - the Medici family, Martin Luther, John Calvin, Issac Newton, Charles Darwin, and Benjamin Franklin, who is credited with having brought to the United States one of the major drugs used in the treatment of gout, colchichine.

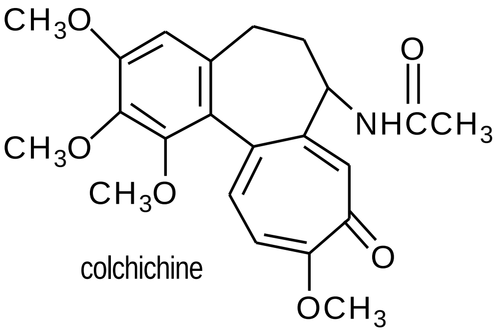
The primary, inherited form of gout is sometimes referred to as **idiopathic** while that form which can be brought on by other disorders such as leukemia or from drug treatment are called secondary.

At one time it was thought that eating rich foods and drinking heavily were the main causes of gout. It is true that a person who is obese is probably involved in a diet high in purine-containing foods such organ meats, herring and anchovies. Such a diet will aggravate a potential gout sufferer. High alcohol consumption will also exacerbate the condition because of the diuresis caused by ethanol (concentrates the uric acid in tissues) and the lactic acid formed from ethanol metabolism (lowers pH).

A condition known as Lesch-Nyhan syndrome is one of the primary causes of gout. An X-linked recessive trait occurring in males, this condition involves a tremendous overproduction of uric acid due to a deficiency of one of the enzymes involved in purine metabolism, hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Other abnormalites lead to mental retardation and aggressive behavior. An obvious symptom of the condition is self-mutilation.

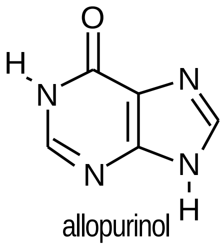
The treatment of gout involves diet and medication. The diet should be low in protein (so as to reduce acidity), provide adequate water, promote weight control, and includes no alcohol. Various antiinflammatory drugs can be prescribed but those which are acids such as aspirin will worsen the situation.

Phenylbutazone, an antiinflammatory, is rarely used because low concentrations inhibit tubule secretion of uric acid. However, if no other drug relief can be found, it may be prescribed.



colchichine

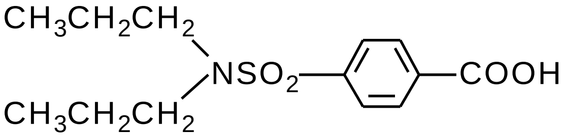
Colchichine is a natural material produced by the autumn crocus and meadow saffron. Its mechanism of action, as far as it is known, is three-fold: it interferes with microtubule-spindle formation in the proliferation of the cells responsible for inflammation; it has an antihistaminic effect; it prevents the release of an inflammatory glycoprotein which neutrophils produce upon phagocytizing urate crystals. Colchichine can be given orally or i.v. but accumulates in the body and becomes toxic. No more than 7 mg can be taken within 48 hours. It is a possible teratogen and produces a number of side effects such as GI upset, peripheral neuritis, rashes, blood dyscrasias (bleeding, bruising, tiredness), lowers body temperature, induces hypertension, and so on. However, its effects on inflammation and swelling are dramatic and it can be extremely effective when given as a prophylactic and for beginning, acute attacks.



allopurinol
(Zyloprim®, Lopurin®, Purinol®)

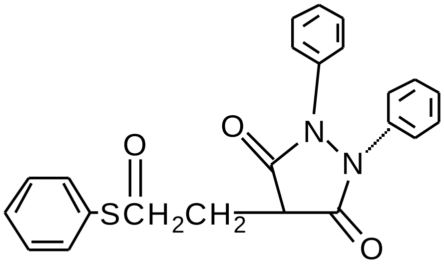
If the inherited defect involves certain enzymes then an enzyme inhibitor could also be effective. **Allopurinol** is a **xanthine oxidase inhibitor**, or purine antagonist, which can reduce the level of uric acid in organs and tissues. Its primary metabolite, alloxanthine, is also active which extends its half-life in the body (half-life of allopurinol is 2-3 hours, for alloxanthine is 18-30 hours). The drug is well-tolerated with few side effects, such as hypersensitivity, which can be accepted. In contrast to colchichine, which is useful in the beginning stages of an attack, allopurinol can itself cause gout inflammations at the beginning of treatment. But these episodes gradually subside. Allopurinol can also interfere with certain cancer treatments. It directly competes with anti-leukemic drugs and lead to increased levels of the anti-cancer agent. In addition it can interfere with the next class of gout drugs, the uricosurics.

The **uricosuric drugs**(having uric acid in the urine) prevent reabsorption of uric acid in the kidney tubules. However, the increased concentration of uric acid in the urine can cause precipitation (renal stones). **Probenecid** is the prototypic drug in this class.



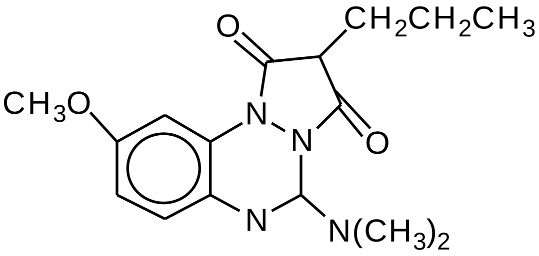
probenecid
(Benemid®, Benuryl®, Probalon®)

Another drug in this pharmacological class, **sulfinpyrazone** was found to have, as a side effect, anticlotting properties in blood which resulted in a 74% reduction in mortality after a heart attack.



sulfinpyrazone
(Anturon®, Anturane®, Zynol®)

Apazone is an analgesic (pain reliever) not currently available in the United States. It is absorbed well through the G.I. tract and binds extensively to plasma proteins thereby extending its half-life (20-24 hours). Its mode of action is to inhibit prostaglandin synthetase. This, in turn, affects the pain centers and decreases the inflammatory response.

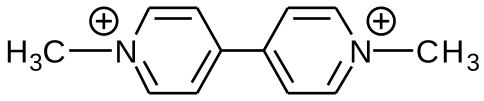


apazone (Rheumox®)

It should be noted that allopurinol and/or uricosuric agents do not replace colchichine in acute attacks of gout because in most cases they don't work as effectively. Therefore they are used more in chronic cases and in combined therapy with cholchicine.

5. Nephrotoxicity

Because just about every kind of molecular species except highly plasma-protein-bound molecules will be forced into the kidney tubules through the cells lining the tubules and then possibly reabsorbed further down the line, drugs and poisons can become concentrated in the cells of the tubules during their passage. Many of these materials can be extremely toxic to the functioning of the active transport systems, carrier proteins and metabolic proteins in those cells. The end result is destruction of the kidney or nephrotoxicity. One such example is the herbicide **paraquat**. In large doses it is acutely toxic to the lungs. But in sublethal concentrations it can reduce its own elimination because it destroys the kidney secretory system. If it is not excreted effectively it will remain in the blood and attack the lungs. Paraquat has been used to spray illegal marijuana fields both within and outside of the U.S.



paraquat

IX. DRUGS AND POISONS AFFECTING THE PERIPHERAL NERVOUS SYSTEM

Although the nervous system is an integrated complex, we will attempt to look at the effects of drugs and poisons on the peripheral system first as this is easier to understand. However, many of the agents we will be discussing also have CNS effects. Those will be mentioned and then covered in more detail later in the section on CNS drugs.

Drugs (poisons) can mimic or block the effects of naturally-occurring neurotransmitters. Therefore, many of them will be agonists or antagonists. Sometimes the action of the drug will be other than that of agonist or antagonist and it's overall effect will be indirect. This is understandable if we look at the various ways xenobiotics might affect the neurotransmitter systems. A drug or poison might

a. stimulate a receptor (agonist)
b. block a receptor (antagonist)
c. prevent the synthesis of a neurotransmitter
d. stop the storage of neurotransmitter in the synaptic vesicles
e. prevent exocytosis
f. enhance exocytosis
g. cause hyperpolarization of a synaptic membrane
h. inhibit the breakdown of a neurotransmitter
i. prevent the reuptake of a neurotransmitter or precursor
j. enhance the binding of a neurotransmitter

ANYTHING ELSE YOU CAN THINK OF?

What effect would each of the modes of action listed above have on cholinergic and adrenergic systems? Think about it. It is a challenging exercise.

In this section we will consider first the drugs and poisons affecting the peripheral cholinergic system and then those affecting the adrenergic system. Within these subdivisions you will look at agonists and antagonists as well as the specific receptors which are involved in drug action.

A. The Cholinergic System (Parasympathetic And, To A Certain Extent, Sympathetic)

1. Cholinergic Agonists (Parasympathomimetics)

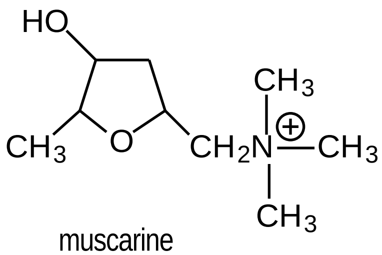
These species will be broken down into those agents impinging upon muscarinic receptors (on neuroeffector cells) and then those complexing with nicotinic receptors (preganglionic, neuromuscular).

a. muscarinic agonists

The muscarinic receptor was so named because of the results of experiments with peripheral nerve cells which were found to respond when

the molecule muscarine was painted on the nerve ending. Appropriately the first drug to be considered will be muscarine. As before the structure of the drug will be given with pertinent information as to source, lethal dose, uses, antidotes and other interesting information.

The ***Amanita muscaria*** mushroom from which muscarine is isolated is also psychoactive. It was believed at first that muscarine was the primary CNS agent. However, more detailed research indicated that muscarine only constituted 0.003% of the fungus. Other species of ***Inocybe*** and ***Clitocybe*** have more muscarine than ***muscaria***. Other isoxazole components of the ***muscaria*** mushroom, such as ibotenic acid and its metabolites, are the main causes of amanita intoxication. This mushroom is believed to have been involved in ancient rituals of the Old World, especially in the Ayrian culture which lived in Siberia around 2000 B.C. This rite worshipped a god called Soma whose presence on earth occurred in the mushroom, ***Amanita muscaria***. Rituals involved brewing a juice with the mushrooms which was consumed by priests. Their urine (isoxazole metabolites) was collected and drunk by others. This ceremony could involve many people and several metabolic rounds until everyone was intoxicated.



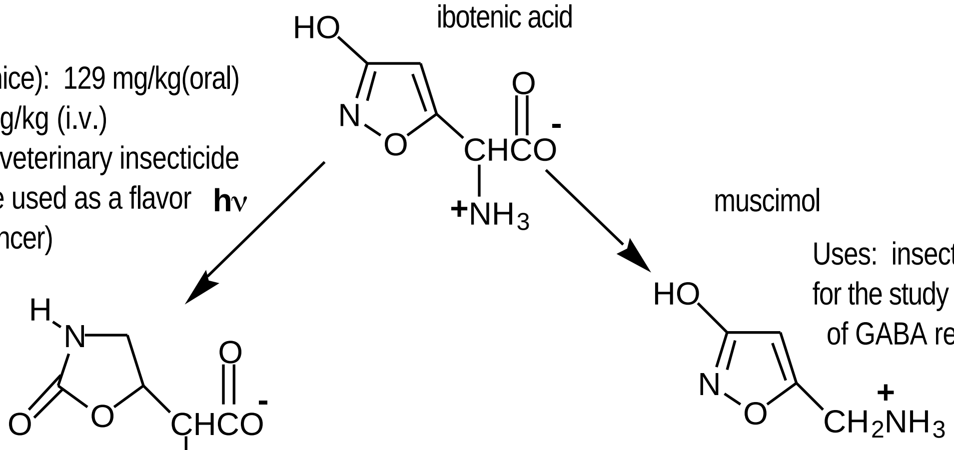
muscarine

Source: mushroom *Amanita muscaria* (fly agaric) found in north temperate climates in wooded areas especially around birch trees

LD_{50} (mice): 0.23 mg/kg (i.v.)
Symptoms: contracted pupils, diarrhea, bradycardia (slowed heart beat), nausea, convulsions, death ("rapid mushroom poisoning")
Uses: cholinergic
Antidote: atropine sulfate

ibotenic acid

LD_{50}(mice): 129 mg/kg(oral)
39 mg/kg (i.v.)
Uses: veterinary insecticide
(once used as a flavor enhancer)



muscazone

muscimol

Uses: insecticidal properties; for the study of GABA receptors

LD_{50}(rats): 45 mg/kg (oral); 0.5 mg/kg (i.v.)
(mice): 3.8 mg/kg (s.c.); 2.5 mg/kg (i.p.)

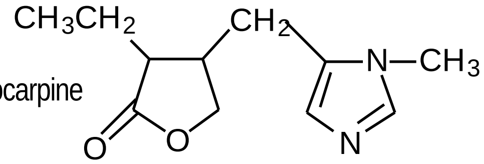
It is interesting to note that the Ayrians were the ancestors of many of the cultures of Asia and the Middle East. Their religion was coalesced with those of conquered Indo-Eurpean beliefs and evolved into Hinduism. The **Rig Veda**, which records some ancient tales of drugs, is a collection of hymns or vedas reminiscent of the Ayrian creed.

Other species of amanita are among the deadliest fungi known. Polypeptide-like toxins in ***Amanita phalloides***, or death cup, can prove fatal or at the very least can cause permanent liver and kidney damage. These mushrooms are common in the temperate climates of Europe and North America. They are responsible for the majority of what is called "slow" mushroom poisoning in the U.S. In fact, it was only a few years ago that the local newspaper reported a case of amanita poisoning within SLO county. Mushroom poisoning is known as **mycetism.** In addition the amanita also contain bufotenine which has CNS effects. See the ASIDE which discusses the peptide poisons.

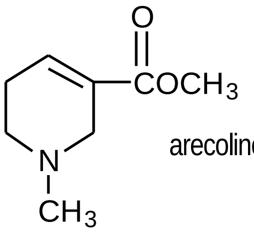
Pilocarpine and **arecoline** are plant products.

Pilocarpine is the active ingredient in Ocusert®, an antiglaucoma agent, which can be inserted behind the lower eyelid.

pilocarpine



Source: leaflets of South American shrub *Pilocarpus jaborandi*
Uses: treatment of open-angle glaucoma
No LD_{50} data but warnings state that it is Poisonous!

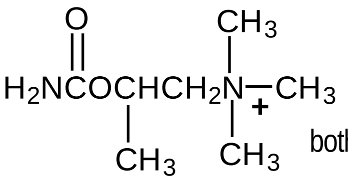


arecoline

Source: betel nuts
LD_{50} (mice, dogs): 100, 5 mg/kg (s.c.)
Uses: euphoretic, antihelmintic (worms)

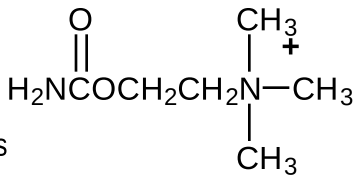
(The picture above was taken by me in Vietnam outside of DaNang city. The betel berries are unripe.)

Betel nut, the source of arecoline, is dried, smoked or salted in preparation for chewing by the natives of southern Asia, Indonesia, the Philippines and eastern Africa. The pieces are rolled in a betel leaf vine which has been smeared with quicklime. Chewing this concoction makes the saliva red. The musical **South Pacific** has Bloody Mary as one of its main characters. Her name denoted the color of her tongue and teeth.



bethanechol

both are as **Cl**$^-$ salts



carbachol

Uses: treatment of kidney problems; restoration of peristalsis after surgery

LD_{50} (mice): 50 mg/kg (oral)
0.3 mg/kg (i.v.)
Uses: treatment of chronic wide-angle glaucoma

Bethanechol and **carbachol** are synthetic muscarine agonists which are fairly persistent in the nervous system because they are not susceptible to breakdown by acetylcholineesterase.

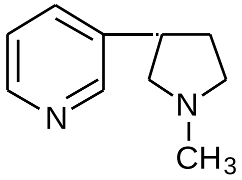
Carbachol also stimulates nicotinic receptors slightly.

b. nicotinic agonists

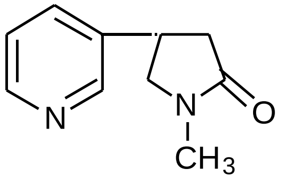
Of course, you have ascertained that the nicotinic receptor was identified as such because nerve cells as well as neuromuscular cells responded to being treated with nicotine. Because of the prevalence of nicotinic receptors in both the parasympathetic and sympathetic nervous systems, you can correctly assume that drugs affecting these sites lack selectivity. In fact, there are no known beneficial uses for nicotinic agonists. To the contrary, nicotine is a POISON.

The principal metabolites of nicotine are cotinine and nicotine 1'-N-oxide. There is some evidence that another product of nicotine oxidation is a quarternary nitrogen derivative which may covalently modify proteins. This

might be the way in which nicotine contributes to cancer. Analyses have shown that passive smokers, especially children and babies, have high levels of cotinine in their blood, indicating that even being in the presence of cigarette, cigar or pipe smoke can be detrimental to one's health.



nicotine



continine
nicotine metabolite

Source: dried leaves of *Nicotiana tobacum* (2-8%) and *rusticum*
LD_{50}(rats): 50-60 mg/kg (oral)
$LD_{minimum}$(dogs): 5 mg/kg
$LD_{minimum}$(human adults): 40-60 mg
Symptoms of overdose: nausea, vomiting, mental confusion, convulsions
Uses: insecticide (Blackleaf 40®)

Nicotine affects the CNS in that it is an addicting drug. (Addiction will be discussed more thoroughly later). Low concentrations are stimulatory while high concentrations cause depression. The CNS and PNS effects include muscle relaxation, facilitation of memory and decreased irritability. Withdrawal symptoms include insominia, irritability, and constipation as well as weight gain. The rate of recidivism (relapse) for those trying to "kick the habit" is similar to that for heroin and alcohol addiction.

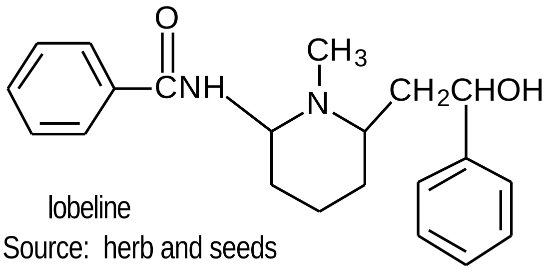
Nicotine-containing gums can be prescribed by physicians and used in conjunction with behavioral modification in the treatment for such addiction. Nicorette® is produced by Lakeside pharmaceuticals, a subsidary of Merrill Dow. It contains nicotine polacrilex, that is, nicotine bound to an ion exchange resin in a sugar-free(sorbitol) chewing gum base with glycerin, sodium bicarbonate and sodium carbonate added (probably for pH control and maximum absorption). The dose in one piece of gum is equivalent to 2 mg of nicotine (one-half the estimated amount for smoking one cigarette per hour). The addiction can be transferred from cigarettes to the gum and so the course of treatment that is recommended is a maximum of three months, although it has been found that it can be used for up to one year without increased use. Within a year of being first marketed (February 1984), about 5 million prescriptions were written. The success rate for its use has been estimated anywhere from 15% to 50%. Of course we also have nicotine transdermal devices - Nicotrol®- and nicotine inhalers. The patches went from prescription to OTC in a very short period of time.

The goal of smoke-free society has been established by the American Medical Association. The Surgeon Generals of the U.S. have more than once publicly denounced smoking and chewing tobacco.

Besides its addictive nature, the tars and other chemicals produced in cigarette smoke, such as benzo(a)pyrene, are carcinogenic. There is an undisputable epidemiological relationship between the incidence of lung

cancer in smokers and cancer of the gums, tongue, lips and jaw in pipe smokers as well as tobacco chewers. 125,000 of 412,000 deaths due to lung cancer have been attributed to smoking. Smoking also exacerbates cardiovascular disease and it is believed that 170,000 out of 565,000 deaths from coronary artery disease are probably due to smoking. Costs to the U.S. in terms of 1985 health care dollars came to more than $16 billion. Indirect costs, such as lost productivity, have been estimated at some $37 billion annually. In addition smoking in bed or a related smoking incident are the leading causes of fire deaths and have been implicated in $500 million in other losses.

American cigarettes have on the average less than 0.7% nicotine while some cigarettes from the Far East contain up to 9% or more. Considering the toxic nature of nicotine the latter could be deadly.



lobeline
Source: herb and seeds of *Lobelia inflata* (Indian tobacco)

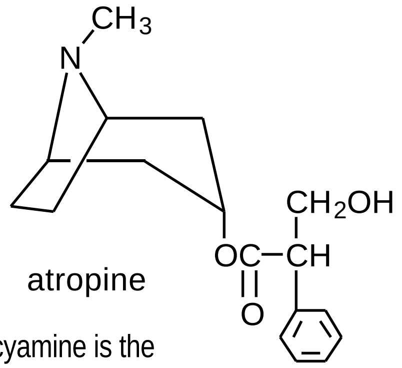
Lobeline is a nicotine substitute with little practical use.

2. Cholinergic Antagonists (Blockers)

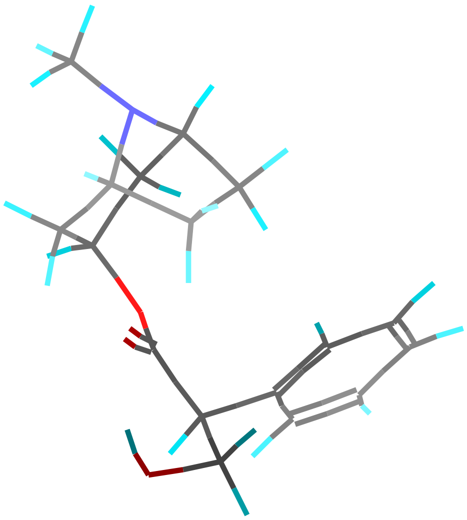
These materials will bind with muscarinic and nicotinic receptors and theortetically should produce no biological cholinergic response. However, some of these agents will depolarize the postsynaptic membrane before blocking it.

a. **antimuscarinics** - Keep in mind that almost every organ and tissue in the body are ennervated by both the parasympathetic and sympathetic systems. If muscarinic receptors are blocked the stimulation to the tissue will be overwhelmingly sympathetic. So the effects of antimuscarinics will appear as sympathetic stimulation.

Atropine and **scopolamine** are alkaloids produced by members of the plant family ***Solanaceae*** which includes belladonna (***Atropa belladonna*** -source of atropine), henbane (***Hyoscyamius niger*** - the source of scopolamine), tomatoes, potatoes, green peppers, eggplant, and members of the Datura subfamily or genus, thornapple, Jimson or Jamestown weed, angel's trumpet, stinkweed, mandrake, and devil's apple. In the eatable plants it is the green portions which are poisonous: vines, leaves, and sprouts.



atropine



hyoscyamine is the (l) isomer

LD_{50} (rats): 750 mg/kg (oral)
The fatal dose in children may be as little as 10 mg

Symptoms: blurred vision, dry mouth agitation, delirium, increased heart rate

Uses: antidote for excessive cholinergic stimulation; tremor treatment; wide angle glaucoma; stomach disorders; preanesthesia

Arco-Lase Plus®-mixture of enzymes, hyoscyamine, atropine, and phenobarbital

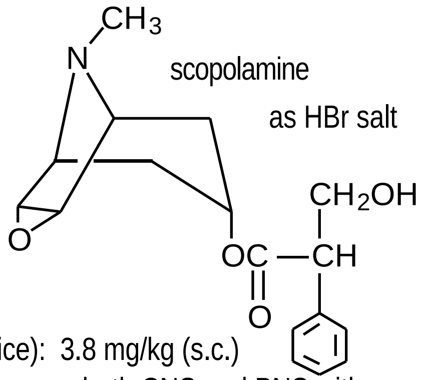
Donnatal®-hyoscyamine, atropine, scopolamine, and phenobarbital

All parts of the belladonna plant contain the alkaloids. Linne named the species after one of the Fates, Atropos, who cut the thread of life. In Italy during the Middle Ages an extract of the plant was dropped onto the eyes in order to dilate the pupils (this practice gave rise to the name "belladonna" or beautiful lady since large dark pupils were considered a mark of beauty). Today atropine analogues, such as homatropine, are still used to dilate the pupils for opthalmalogical studies. Sympathomimetics can also be used for this purpose. See the Aside on “Eye Openers”.

The solanaceae alkaloids and other other sources of antimuscarinics affect the CNS. They can produce hallucinations in addition to their effects on the peripheral nervous system. Witchcraft of the Middle Ages produced mixtures of plants - deadly nightshade, monkshood, and hemlock among them - as "flying ointments". The combined toxins disturbed the rhythm of the heart and led to delirium which could create a sensation of rising and falling, that is, flying.

Atropine is an "antidote" to poisoning by excessive stimulation of cholinergic muscarinic receptors such as occurs in mucarine and organophosphate insecticide poisoning.

Vinegar, acetic acid, has been mentioned as a home remedy, an antidote, for atropine poisoning. What basis in fact might this antidote have?



scopolamine

as HBr salt

LD50 (mice): 3.8 mg/kg (s.c.)

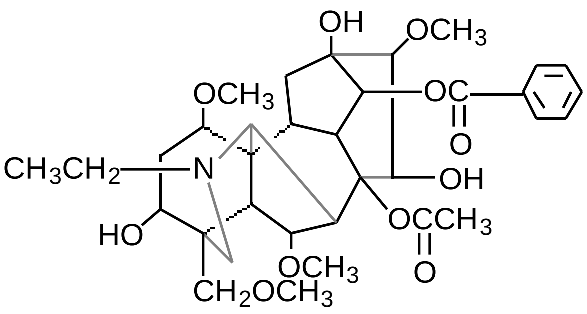
Symptoms are both CNS and PNS with scopolamine; can limit to PNS by methylation of N (methscopolamine)

Uses: antinausea drug (Transderm-Scop®) antihistamine (meth- with phenylephrine and chlorpheniramine - Extendryl®, Histaspan-D®)

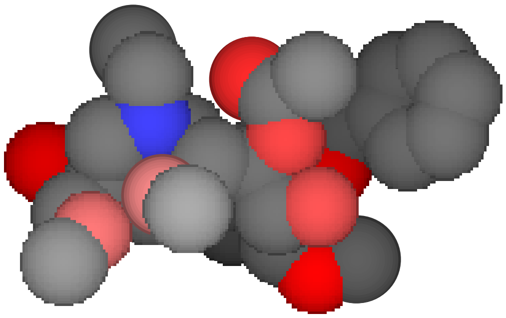
These compounds have been used for many centuries and still find use today in their original forms or through synthetic analogs. Conditions which respond to muscarinic antagonists include irritable bowel syndrome, ulcers, diarrhea, glaucoma, acute rhinitis in addition to some CNS disorders. For example, Lomitil® is a prescription treatment for diarrhea which is composed of atropine and diphenoxylate (an opiod with similar effects).

The most popular use of scopolamine today is for the prevention of motion sickness via the Transderm-Scop® patch. This device delivers about 0.5 mg of the drug over a three day period. Scopolamine and other drugs in this class can accumulate in significant quantities in the blood and some can cause CNS effects such as drowsiness, dry mouth and blurry vision. In rare cases amnesia and even psychosis can occur. The sedation and dry mouth are useful effects in that some of the compounds are used as tranquilizers and to dry up the mouth prior to anesthesia.

Aconite is another alkaloid which is found in members of the ***Ranuncullaceae*** family: monkshood, friar's cowl, mousebane, wolfsbane, delphinium. Monkshood was a mainstay of poisoners and witches throughout history and was believed to be a protection against werewolves. It has been used for millenia as a potent arrow poison.



aconitine

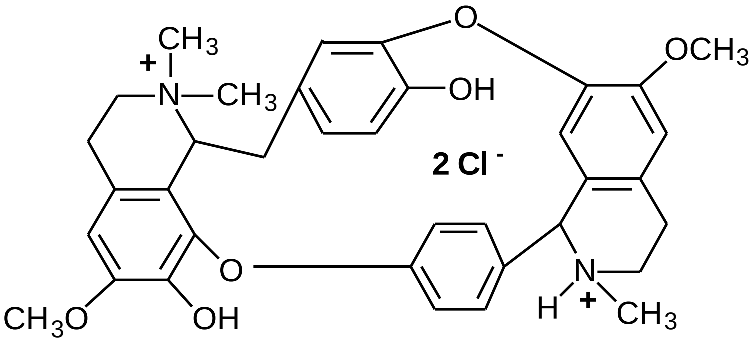


LD50 (mice): 0.166 mg/kg (i.v.)
0.328 mg/kg (i.p.)
~ 1 mg/kg (oral)

monkshood

b. antinicotinics - Remember that nicotine receptors can be found at the postsynaptic membranes of preganglionic synapses in both the sympathetic and parasympathetic systems as well as at neuromuscular junctions. Drugs may be fairly nonspecific in their attack on these receptors or may actually have some specificity for receptor subtype.

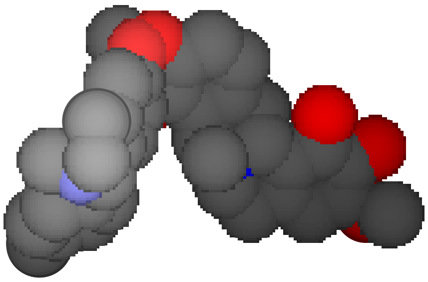
The first category of antinicotinics are those which cause neuromuscular blockade, that is, muscle paralysis. They do so by stabilizing the membrane to depolarization. These drugs affect both the PNS and SNS and do not cross the blood-brain barrier. Look and their structures and see if you can determine why this is so. With these properties they are excellent agents for use in muscle relaxation prior to surgery.



(+) tubocurarine chloride

(Tubadil®, Delcurarine®, Curarin-HAF®)

Source: active agent derived from the bark of various subspecies of South American *Strychnos* and *Chondrodendron*.
LD_{50} (mice): 0.63 mg/kg (i.p.), 0.7 + 1 mg/kg (s.c.)
Uses: arrow poison of South American Indians of the Amazon and Orinoco Valeys; muscle relaxant

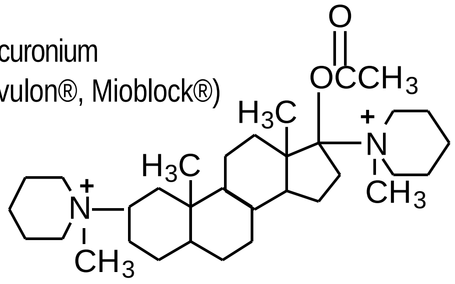


D-tubocurarine or **curare** is a natural alkaloid used by South American natives as an arrow poison. Since it paralyzed the extremities first, the

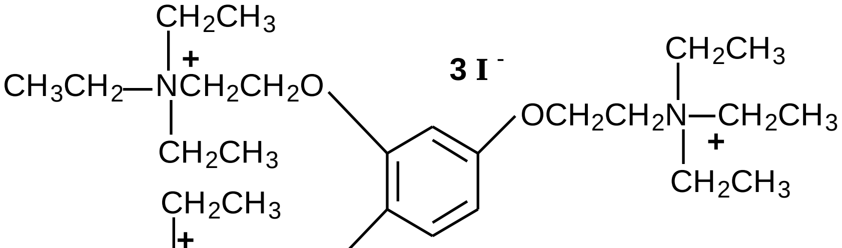
victim was conscious of his fate until respiratory paralysis or some other trauma ended his life.

Pancuronium is a drug used to paralyze patients undergoing intubation, that is, being put on a respirator.

pancuronium
(Pavulon®, Mioblock®)



LD_{50} (mice): 0.047 mg/kg (i.v.)
0.152 mg/kg (i.p.)
0.167 mg/kg (s.c.)
21.9 mg/kg (oral)
(rats, rabbits): 0.153, 0.016 mg/kg (i.v.)

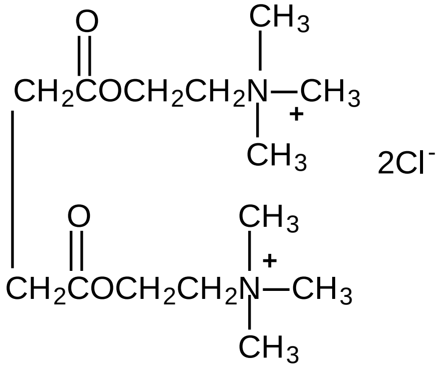


gallamine

(Retensin®, Relaxan®, Flaxedil®)

Gallamine is a synthetic muscle relaxant as are several analogs of curare.

Succinylcholine is a depolarizing blockader. It depolarizes (stimulates) the membrane and then blocks (antagonizes) it. This dual action is not uncommon in drugs. Such activity is termed agonist-antagonist.

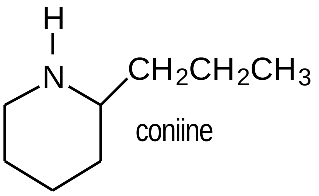


succinylcholine
(Anectine chloride®, Ultrpal chloride®)

LD_{50}(mice): 0.45 mg/kg (i.v.)
Uses: short duration skeletal muscle relaxant

Coniine is a poison found in the spotted hemlock, ***Conium maculatum***. This type of hemlock is found commonly throughout the United States and the world, including SLO County. White disperse flowers on a long purple stalk are routinely mistaken for its nontoxic look-alike - the edible wild parsnip. It was a hemlock brew which Socrates

ingested in 399 B.C. as his capital punishment for treason. His death by slowly creeping paralysis was recorded by Plato, his student.



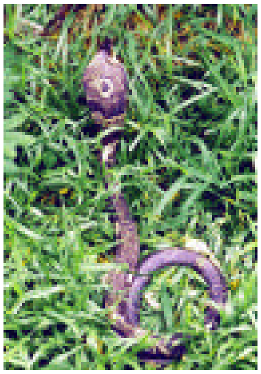
coniine

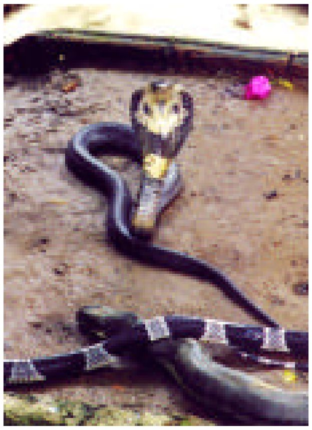
Source: spotted hemlock
There are two other alkaloids in this plant - conia and conhydres
Symptoms: salivation, weakness, paralysis, of sensory and motor nerves, asphyxia

The venom of the elapid snake ***Bungarus multicinctus*** (Southeast Asian banded krait) contains α**-bungarotoxin**, a protein composed fo 74 amino acids (about 8000 daltons) having five disulfide bridges. The LD_{50} in mice is 0.21 mg/g (s.c.)

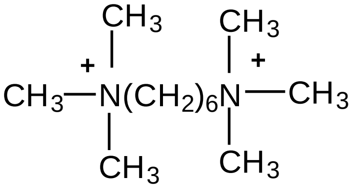
Cobra toxin (cobrotoxin), a 62 amino acid protein with four disulfide bridges, causes nondepolarizing blockade at neuromuscular junctions.

Pictures taken at a snake farm in Vietnam.



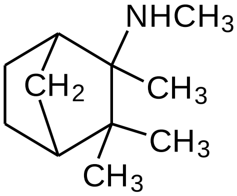


Agents which cause antagonism of preganglionic nicotinic receptors are of little use because of their nonselectivity. However, there are times when such drugs are needed such as for the treatment of a hypertensive crisis or to produce hypotension during a surgical procedure. Hexamethonium and mecamylamine are two such molecules.



hexamethonium

(Gangliostat®, Esametina®, among others)



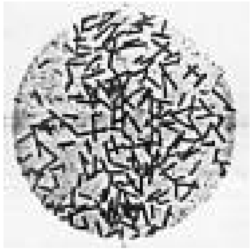
mecamylamine

(Inversine®, Mekamine®,Revertina®)

3. Agents Affecting The Availability Of Acetylcholine

Recall that there are many possibilities for drug action in the nervous system besides agonism and antagonism. We will now consider species which can enhance or deplete the concentration of a neurotransmitter in the synaptic cleft.

a. Botulism toxin is a series of protein exotoxins produced by the anaerobic, gram-negative bacterium ***Clostridium botulinum***. There are seven strains of botulinum each of which produces specific toxins (A, B, C_a, C_b, D, E, F, and G). Types A, B, E and F cause food poisoning in humans. The toxins are readily absorbed through the mucous membranes of the stomach, intestines and respiratory tract. Poisoning is always acute. One of the most toxic materials known, the lethal dose for humans is 10^{-9}mg/kg in its unconcentrated form. Considering the molecular weight of the protein that amounts to 3.3×10^{-17} mole/kg. By comparison, cyanide poisoning occurs at a level of 2×10^{-4} mole/kg. (See the Aside on Cyanide.)



The spores of the bacterium can be found in the soil and grow in the absence of oxygen. The proteins are heat-labile and can be destroyed by heating the food to the boiling point of water for a period of time and properly canning it. The spores are also sensitive to NaCl and will not grow in NaCl solutions of greater than 10%.

Botulism toxin binds to the presynaptic membrane of cholinergic neurons and prevents the exocytosis of acetylcholine. The symptoms of poisoning include difficulty in swallowing, speaking, moving, and focusing the eyes, paralysis of the extremities quickly reaching the trunk and leading to respiratory paralysis and death. Poisoning is difficult to diagnose but can be treated with antisera (immune system treatment). This type of poisoning has a 50% mortality rate. Because of its easy penetration of membranes, the toxin can enter the brain through the blood-brain barrier and cause permanent brain damage to survivors.

In 1991 the FDA approved the use of botulism toxin (BT) for the treatment of an "orphan" condition called blepharospasm. A victim of this syndrome will experience uncontrollable winking caused by spasms of the eyelid muscles. Use of botulism toxin will cause abatement of the symptoms in 90% of patients for 2 to 4 months.

BT has also been tested for the relief of crossed eyes (strabismus) in children where it relieved that condition for two years in more than 50% of those treated.

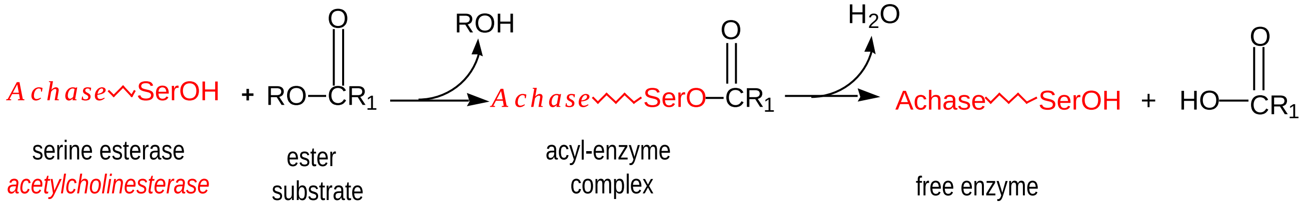
Experimentation is proceeding using BT for the relief of spasms of the face and neck, voice disorders involving laryngeal spasms as well as stuttering. (**Harvard Medical School Healthletter** February 1991)

b. Black widow spider venom contains a labile protein toxin, **α-latrotoxin**, as well as other components which aide in the attack on the victim. The LD_{50} (mice) is 0.18-2.20 mg/g. The toxin causes clumping of synaptic vesicles at the presynaptic membrane causing premature release of acetylcholine. As you might conjecture this produces a muscle spasm and pain at the site. This pain and spasm spreads to the chest, abdomen, and joints. Salivation, sweating and nausea accompany the symptoms already listed. The lethal reputation of the black widow spider pertains mostly to her mate. Of the approximately 500 bites reported in the U.S. per year, less than 1% are fatal. The very young and infirm are the most susceptible to a fatal response. Recovery usually beings in 12-24 hours and is complete in one week. An antivenom is available for immediate treatment.

c. β-bungarotoxin is another toxin produced by the Southeast Asian krait ***Bungarus multicinctus***. It is a protein with about 180 amino acids and is made up of several subunits. The LD_{50} (humans?) is 0.019 mg/kg (i.p.) The mode of action of this toxin is to promote premature release of acetylcholine. The venom also contains a phospholipase A_2 which aides in membrane destruction.

d. anticholinesterases. An effective way to prolong the activity of a neurotransmitter in the synaptic cleft is to prevent its breakdown. Since the primary mode for cessation of acetylcholine (Ach) activity at the postsynaptic receptor is through hydrolysis by acetylcholinesterase (Achase), inhibition of Achase will make the Ach prolong its stay at the receptor. The agents, both drugs and poisons, which cause such inhibition are called anticholinesterases.

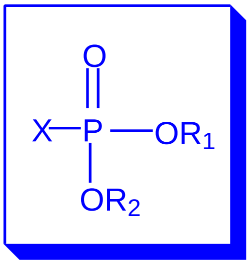
The mechanism of action of anticholinesterases is to form a stable covalent complex with the Achase enzyme. Achase is one of several enzymes known as serine esterases. Other examples include the intestinal enzymes trypsin and chymotrypsin as well as the blood clotting agent thrombin. During the course of the catalysis the alcohol -OH of a serine side chain in the active site of the enzyme forms an ester complex, called the acyl-enzyme, with the substrate. So, acetylcholine will go through similar chemical reactions with Achase.



Anticholinesterases also react with Achase but the intermediate ester formed will not hydrolyze easily. As a result the enzyme is tied up irreversibly with the inhibitor and the Ach can go on working at its receptors with impunity.

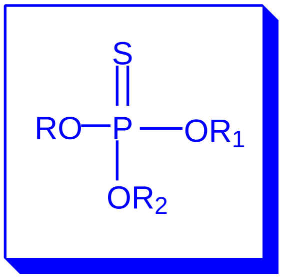
Anticholinesterases fall into two major organic categories, the organophosphates and the carbamates. We will consider each class and its uses.

Organophosphates are esters of phosphoric acid. They have the general structure illustrated below.



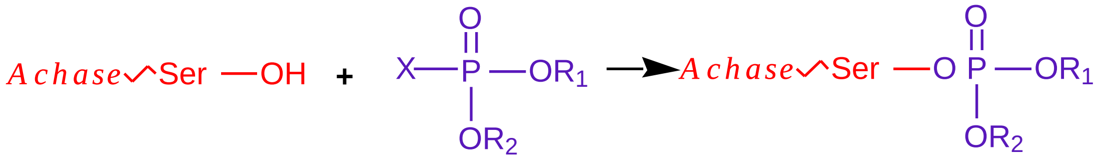
The general structure of organophosphates. The X is a good leaving group. In the prototype compound DIFP, diisopropylfluorophosphate, F was used.

If one or more of the ester positions is held by an R group, the compound will be resistent to detoxification and make a more stable derivative of Achase.



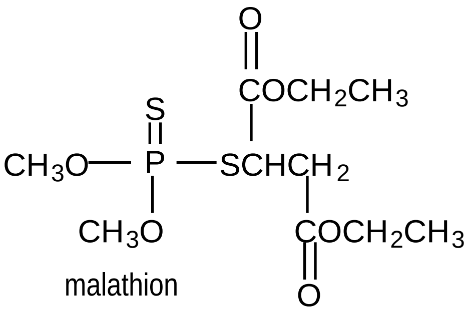
Organophosphate insecticides are designed for nontoxicity to humans in that the S is relatively nontoxic. Toxicity is achieved by the host's oxidation of the S to O as well as the inability to detoxify by hydrolysis of the ester groups.

The phosphate derivative can form a stable complex with the active site serine of Achase. Should chemical breakdown of the phosphate ester groups occur before the complexation, nontoxic products will be formed. The presence of alkyl groups directly attached to phosphorous stabilize the phosphoryl-enzyme derivative. As you will see when we discuss nerve gases, some of the products released during the complex formation can be deadly in their own right.



acetylcholinesterase organophosphate stable enzyme-phosphate complex

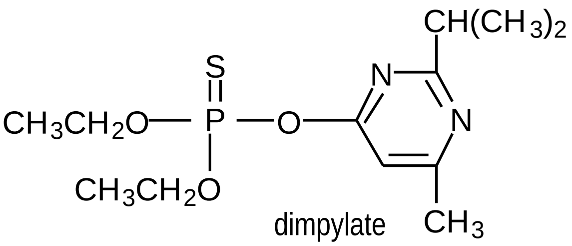
Organothiophosphates find widespread utility as pesticides. You may recognize the brand names of some of the following compounds.



malathion

(Cuthion®, Malamar®, etc.)

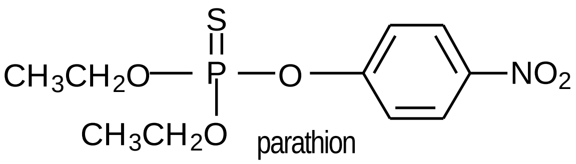
LD_{50}(rats): 1.38 mg/kg(oral)
>4.44 g/kg(dermal)



dimpylate

(Diazinon®,Spectracide®, Garden Tox®, etc.)

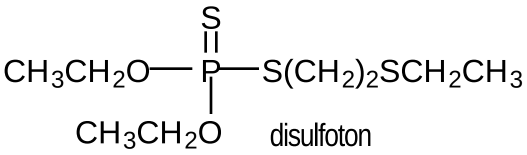
LD_{50}(rats): 108 mg/kg(oral)
200 mg/kg(dermal)



parathion

(Alleron®, Thiophos®, etc.)

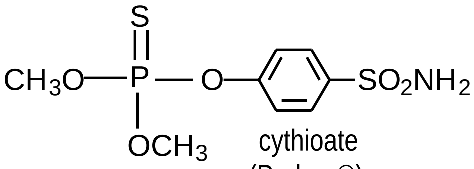
LD_{50}(rats): 13 mg/kg(oral)
21 mg/kg(dermal)



disulfoton

(Dithiosystox®, Solvirex®, etc.)

LD_{50}(rats): 6.8 mg/kg(oral)
15 mg/kg(dermal)



cythioate
(Proban®)

systemic flea control for dogs and cats
LD_{50} (rats): 160 mg/kg (oral)

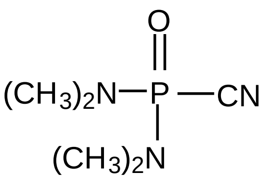
Parathion is the material of this type most frequently involved in fatal human poisonings. 0.1 mg/kg can kill a 5-6 year old child.

Recall in our discussion of routes of biotransformation we considered species differences using malathion as an example. Insects convert this compound to its toxic oxidation product more quickly than they detoxify it by hydrolysis. Humans do the conversions in the opposite priority. However, the insects which might be different from the general population and perform detoxification reactions at a faster rate would survive pesticide application and their "resistant" genes would be selectively passed on to the next generations.

Unfortunately the history of organophosphorous insecticides began with the search for substitutes for the war gases of World War I. This aspect evolved to the production of deadly nerve gases which are being stockpiled by the major powers and were used by the minor ones (Russia-Afghanistan, Iran-Iraq). It was this frightening potential which was a target in the Desert Storm operation.

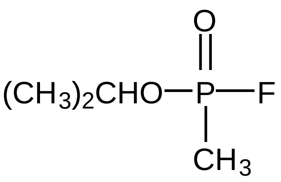
The U.S. has been trying to develop a "binary weapon" for years. Chemical precursors of nerve gases are housed in an explosive device ("Big Eye" bomb) and the detonation forms the gas.

Chemical Warfare Agents



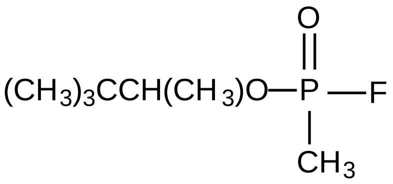
tabun (GA)

LD(human): may be 0.01 mg/kg
LD_{50}(mice): 0.6 mg/kg(i.p.)



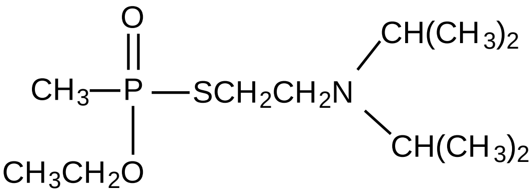
sarin (GB)

LD(human): may be 0.01 mg/kg
LD_{50}(mice): 0.42 mg/kg(i.p.)



soman (GD)

LD(human): may be 0.01 mg/kg
LD_{50}(mice): 0.62 mg/kg (i.p.)
0.78 mg/kg (dermal)



VX

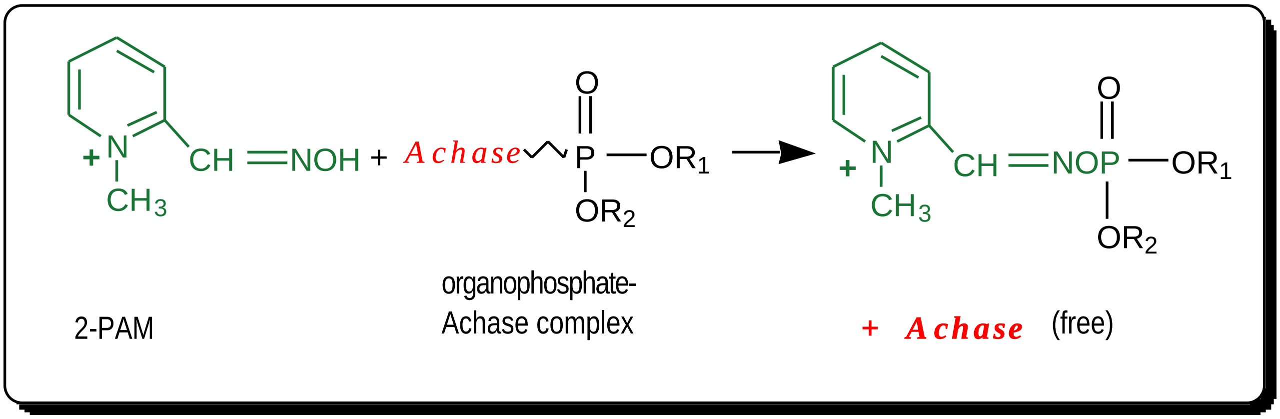
LD_{50}(mice): 70.0, 76.7 mg/kg (male, female-i.p.); (rabbits): 15.4 mg/kg (s.c.)

Containers of VX stored in Indiana

Symptoms of poisoning are excessive salivation, pupil constriction, chest tightness, wheezing, diarrhea, nausea, bradycardia, urination, convulsions, eventually death due to respiratory collapse.

Antidotes to nerve gas poisoning are atropine and pralidoxime (2-PAM). The atropine is merely a stop-gap measure to block the fatal Ach stimulation. Soldiers being trained to work in the field contaminated with nerve gas carry atropine- and PAM- containing syringes and hope that they will be able to administer the drug before being overcome. Note that the Achase is still inhibited and the enzyme will actually have to be replaced biosynthetically in order so that the system can operate normally again. You have also treated one type of poisoning with another.

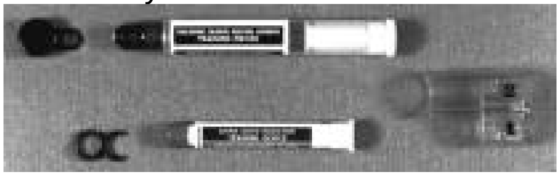
2-PAM is a true antidote in that it forms a complex with the phosphate. This treatment is usually given by qualified personnel in a medical facility. The tradename for the hydrochloride salt is Protopam Chloride®.

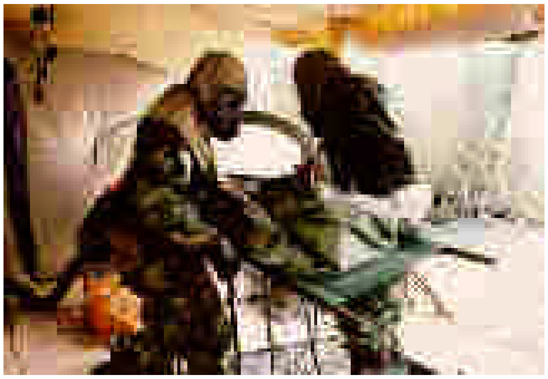


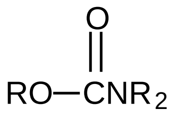
Farm workers are especially susceptible to organophosphate insecticide poisoning. Unsafe spraying conditions can lead to accumulated toxicity and chronic symptoms. Since there are many enzymes in the body which are in the serine esterase family along with Achase and can complex

with these compounds, blood tests can be run to determine the extent of exposure.

In the early 1980s an enzyme was found in squid nerve cells which could potentially detoxify soman and sarin. It was hoped that development of its use might lead to a method of safely destroying nerve gas stockpiles or as an antidote to poisoning in warfare. Not much more has been published about this enzyme. Then during the summer of 1989 it was reported that researchers at Texas A & M had isolated a gene for an organophosphorus anhydrase from a soil bacterium, ***Pseudomonas diminuta***, which might be useful in destroying pesticide residues and nerve gases. They were successful in inserting the gene into insect cells in order to amplify production of the enzyme.



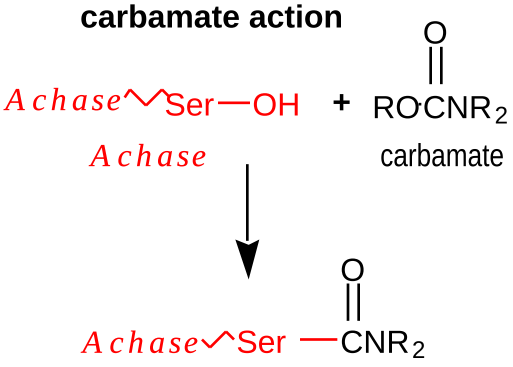




general structure of carbamates

Carbamates will also form a stable complex with Achase but the complex is not as long lasting as that of the organophosphates. Therefore carbamates are not as persistently toxic, although they can still be fatal depending upon dose, site of exposure and other factors previously described.

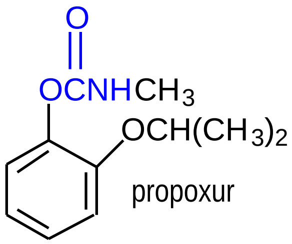
Mechanism of carbamate action



stable complex

Carbamates are also used as insecticides and again, their trade names are probably familiar if you have done any serious gardening and pest control. The use of these compounds is restricted to certain parts of the growing cycle and specific non-food produce. Notice their low degree of dermal toxicity. Carbaryl, for example, is used topcially in some countries to get rid of head lice. It was the misuse of Temik® several years ago on watermelons in California which caused a severe problem and recall of the product. The poisoning was not from the Temik itself but from one of its oxidized metabolites.

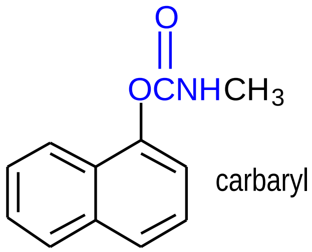
The antidote for carbamate poisoning is atropine only. 2-PAM will stabilize the enzyme-carbamate complex and so exacerbate the poisoning.



(Baygon®, Invisi-Gard®, Suncide®, Unden®)

LD_{50}(rats): 83mg/kg(oral)

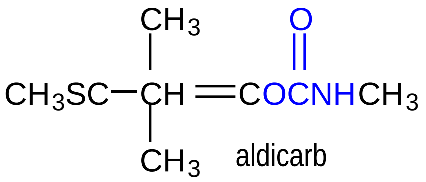
2.4 x10^2 mg/kg (dermal)



(Sevin®, Arylam®, Dicarbam®, Ravyon®)

LD_{50}(rats): 850 mg/kg(oral)

>4 x 10^3 mg/kg (dermal)



(Temik®)

LD_{50}(rats): 0.8 mg/kg(oral)

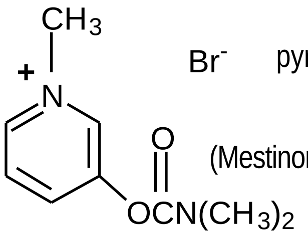
3.0 mg/kg (dermal)

The toxicity of these materials has led to the development of alternative means of insect control. Pyrethrins are natural insecticides produced by plants to ward off predators. Over the past decade or so, more of these natural defenses called allelochemicals have been investigated in the hopes of using them commercially. See the ASIDE on pyrethrins.

Other types of insecticides have been developed and used over the past several decades. To discuss them all requires an entire course . There is an ASIDE which illustrates the halogenated hydrocarbons most of which are not in current use because of their effects on the environment (DDT) and toxicity to humans (Dieldrin/Aldrin). Estrogenic acivity of DDT led to fatal fragility in the eggs of certain predatory birds. All of these compounds are lipophilic and interfere with nerve signal transmission.

Clinical anticholinesterases are mainly carbamates which have use for the treatment of conditions in which Ach stores are depleted or are ineffective. One, physostigmine, is a natural compound used in ancient judgement rituals in West Africa. To judge the veracity of an accused person, he was made to drink a brew made from the Calabar bean. If he got sick and vomited, thus eliminating the poison, he was innocent. If the stuff stayed down and he died, he was, of course, guilty. Physostigmine has an ***in vivo*** duration of inhbition of 3 to 4 hours.

Most of these substances are hydrolyzed more slowly than acetylcholine and so act as competitive substrates for Achase. Edorphonium is simply a competitive inhibitor since it cannot undergo hydrolysis.

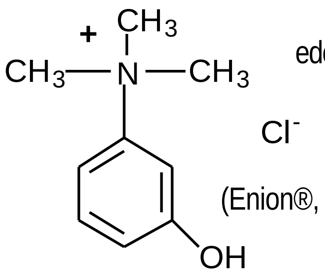


pyridostigmine

(Mestinon®, Regonol®)

Source: synthetic

Uses: treatment of myasthenia gravis (has fewer side effects -salivation, GI disturbances, bradycardia- than neostigmine); antidote to nondepolarizing blockade caused by muscle relaxants during surgery.

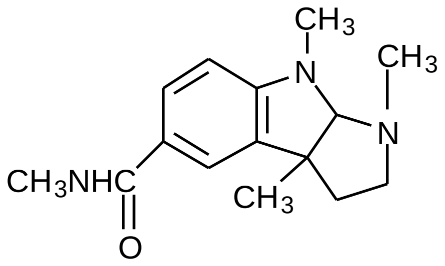


edorphonium

(Enion®, Reversol®, Tensilon®)

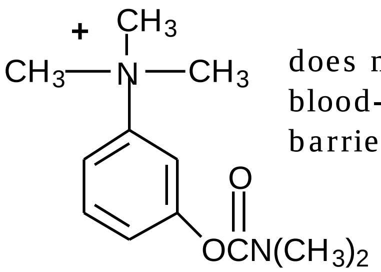
Source: synthetic

Uses: diagnosis of MG, treatment of MG crisis, antidote to muscle blockade for surgery (does not work with succinylcholine and decamethonium)



physostigmine(eserine)
(Antilirium®, Physostigmine salicyclate)

Source: Calabar (ordeal) bean of West Africa
LD (mice): 3 mg/kg (oral)
Symptoms: salivation, bradycardia, seizures, increase in blood pressure
Uses: antidote to atropine poisoning, antidepressant, antihistamine preps.



does not pass blood-brain barrier

neostigmine
(Prostigmin®, Neostigmine Methylsulfate)
(Br^-)

Source: synthetic
LD_{50}(mice, rats): 0.3 mg/kg (i.v.)
0.4 mg/kg (i.m.)
Uses: treatment of myasthenia gravis

Notice that several of the drugs above are used in the diagnosis or treatment of a condition known as **myasthenia gravis (MG)**. MG is an autoimmune disease in which the body's own immune defense system has turned against itself. The targets are the Ach receptors in nerve and neuroeffector cells. As a result the neuromuscular junction begins to deteriorate and there is a rapid loss of muscle tone with exertion. It affects the muscles of the face and throat especially. One may notice drooping eyelids (often held open by adhesive tape), double vision, and facial weakness. As other muscles are affected, breathing may be impaired. The disease occurs most often in young women and men over 60.

B. THE ADRENERGIC SYSTEM

We will proceed with this neurotransmitter system much as we did with the cholinergic system. Keep in mind that the main neurotransmitter here is norepinephrine and that adrenergic neurons are found in the sympathetic (fight or flight) portion of the autonomic, peripheral nervous system.

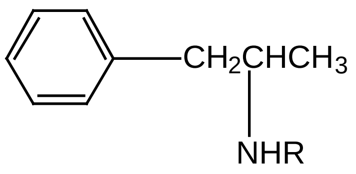
The principal modes of actions of drugs and poisons we will consider will be agonism or pseudoagonism, antagonism, and enzyme inhibition. I use the term pseudoagonism because many of the molecules you will look at prevent the reuptake of norepinephrine into the pre-synaptic neuron, which is the principal means of terminating neurotransmitter action in adrenergic systems. This will result in excess NE in the synapse and increased stimulation of the postsynaptic receptor. Hence the agent is not itself an agonist but rather enhances the natural agonist.

Because of the proliferation of adrenergic synapses in the CNS and the permeability of many of the following drugs into the brain tissue due to inherent lipophilicity or transport systems, several will be psychoactive as well as peripherally active.

1. Adrenergic agonists (sympathomimetics)

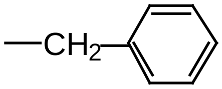
These compounds cause general stimulation of both α- and β- receptors.

a. Amphetamines have a great deal of structural similarity to norephinephrine. Keep this in mind when you are correlating the structures to their activities.



R = -H ; amphetamine

R = $-CH_3$; methamphetamine

R = 

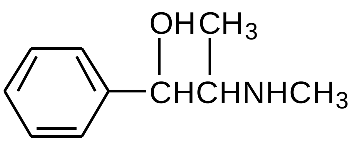
; benzphetamine

Other names for amphetamine include dexedrine (d-form), benzedrine (d,l), phendrine, psychodrine.

Amphetamine, its derivatives and isomers are used mainly for their CNS effects which will be discussed later. They were once the drug of choice for appetite suppression(PNS and CNS effect) and were used for asthma. Effects related to their peripheral activity are anorexia, increased blood pressure, nausea, vomiting, diarrhea, and cardiac arrhythmias.

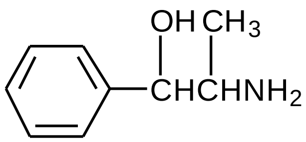
Amphetamine was once used in nasal inhalers but was banned in 1959 because abusers removed the inner filling and chewed it. Now propylhexedrine, with about half the potency of amphetamine, is used. This still causes problems in that some desperate souls will leach the compound from the inhaler's cotton wick and inject the extract (called "crank"). This presents all kinds of problems not the least of which is what happens if a piece of cotton is injected by accident.

Ephedrine, originally isolated from the Chinese plant ***Ma Huang***, causes general alpha and beta receptor stimulation as well as enhances the release of NE. Besides having CNS effects, it can be used as a bronchodilator.



ephedrine

Notice that ephedrine has two chiral carbons. This would give rise to four possible optical isomers- two pairs of enantiomers and four sets of diastereomers. One of the diastereomers is called pseudoephedrine. Both compounds are used widely in over-the-counter decongestants.



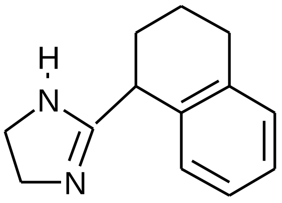
phenylpropanolamine

b. Phenylpropanolamine is another popular OTC ingredient used both for its appetite suppressing qualities and for its decongestant properties. The following is only a partial list of decongestant preparations containing this drug: A.R.M, Bayer Decongestants, Breacol, Combtrex, Congestprin, Contac, Coricidin, Coryban-D, CoTylenol, Covangesic, Dristan, Formula 44-D, Hold, Novahistine, Ordinex, Ornacol, Ornex, Robitussin, Sine-Aid, Sine-Off, Sinutab, Triaminic. Most of these products are a combination of ingredients one of which is an antihistamine. See the aside with a discussion of antihistamines used in the treatment of immune responses and ulcers.

OTC products containing ephedrine		OTC products containing pseudoephedrine	
Bronkaid®	Bronkotabs®	Actifed®	Afrinol®
Ephedrol®	Marax®	Dimacol®	Drixoral®
Nyquil®	Quibron®	Neobid®	Novafed®
Quibron Plus®	Tedral®	Sudafed®	

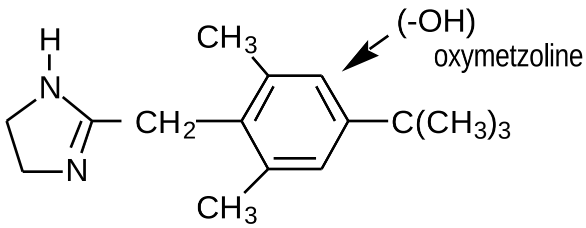
Common side effects of ephedrine	Common side effects of pseudoephedrine
nervousness, headache, rapid heartbeat	agitation, insomnia

c. Tetrahydrozoline, oxymetazoline and xylometazoline are sympathomimetics which can be found in eye drops ("get the red out") and decongestants. Because of their ability to cause vasoconstriction in peripheral blood vessels they can relieve swelling in the vessels of the mucous membranes and the eyes.



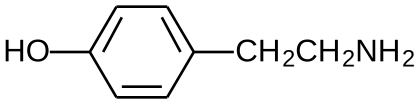
tetrahydrozoline

(0.05% ophthalmic- Visine®, Murine Plus®; 0.05-0.10% nasal- Tyzine®)

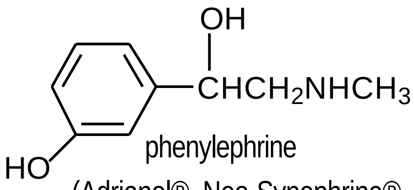


xylometazoline
(Octrivin®, Neosynephrine II®)
(Afrin®, Dristan®, Duration®, 4-Way®, NTZ®, Neosynephrine12-hour®, Nostrilla®, Sinex® - all are"long-acting" nasal sprays)

The natural adrenergic stimulant tyramine was discussed in Part One in the section on enzyme inhibitors. If you will recall, certain foods such as ripe cheeses, red wine and herring contain tyramine. You may add to the list of sources mistletoe and ergot (rye fungus-which is the source of some other interesting stuff). If someone is taking monoamine oxidase (MAO) inhibitors, they could have a hypertensive crisis after ingesting tyramine-containing foods.

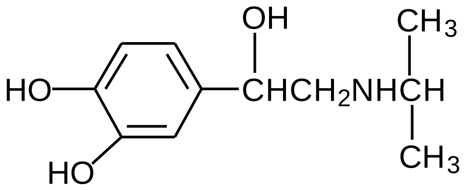


tyramine
(Mydrial®, Uteramin®)



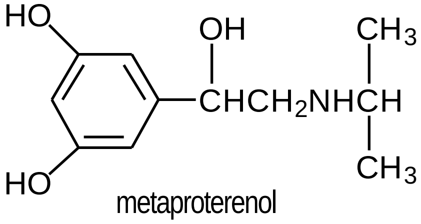
phenylephrine
(Adrianol®, Neo-Synephrine®, etc)

Phenylephrine is a specific α-agonist with very little effect on β-receptors. The other name for this drug, a brand name, is Neosynephrine® and it can be found in nasal decongestant sprays and as a dilator in opthamology. It can also be used with the antihypertensive drug phenoxybenzamine and the smooth muscle relaxant papaverine in order to treat impotence in males.

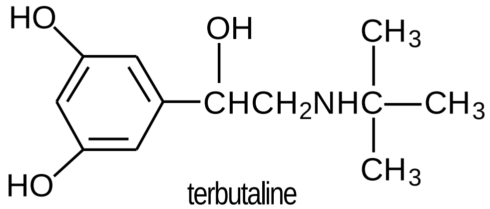


isoproterenol
(Medihaler-Iso®, Norisodrine®)

There are some drugs which are distinctly β-agonists. Their main actions will occur with cardiac tissue, the smooth muscles of the bronchi, blood vessels in muscles, and alimentary tract. If the molecule has certain number of molecular descriptors it will have almost exclusively β_2-receptor activity, which means its effects will be primarily those of bronchodilation with little effect on heart rate. Isoproterenol is a general β-stimulator while the other three, metaproterenol, terbutaline and albuterol, are β_2-stimulators. All of these

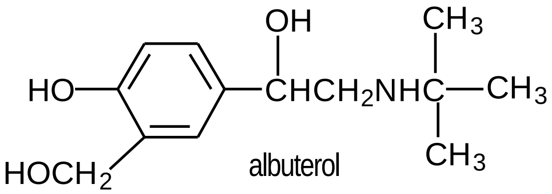


metaproterenol
(Alupent®, Metaprel®)



terbutaline

(Brethaire®, Brethine®, Bricanyl®)



albuterol

(Proventil®, Ventolin®)

compounds are used in bronchial inhalers for the treatment of asthma. They differ in the duration of action. That of isoproterenol is very short and metaproterenol is both less selective and has a short duration. Pirbuterol and bitolteral are also found in the anti-asthma armory. The latter is converted to colteral (also active) in the lung. It is not frequently prescribed because it has a bad aftertaste.

Asthma is defined as reversible airway disease. It affects more than 10% of children and a good number of adults. The drugs shown here were and are used to dilate the bronchi during an asthma attack. However, much has been learned over the past few years about the causes of bronchial constriction and irritation during an episode. Inhaled steroids are the drug of choice to reduce the bronchi inflammation and rapidly lead to asthma relief.

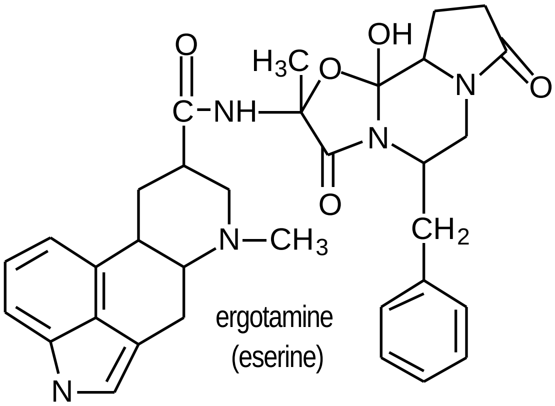
Note that other sympathomimetics can also be used as bronchodilators, but many will have sympathetic side effects. Epinephrine, the sister to norepinephrine, can be found in Primatene® and Bronkaid®, for example, for intermittent problems.

2. Adrenergic antagonists

Since there are a plethora of adrenergic receptors there can be different types of blockade. It is difficult to have an exclusive type of antagonism but some of these drugs come very close.

a. Ergotamine is one of several ergot alkaloids isolated from grain fungus, mostly notably that which grows on rye, ***Clariceps pupurea*** . Ergotamine blocks a-receptors and has actions at dopaminergic and tryptaminergic neurons as well. For centuries ergot has been used in folk medicine during childbirth in order to stimulate contractions and then stem the flow of blood. The uterine stimulation properties also made this material an abortifacient. The vasoconstriction can be toxic because it eventually cuts off circulation to the extremities. The effects will include burning sensations referred to in earlier times as Holy Fire or St. Anthony's Fire. Gangrene can set in with ensuing bloodless loss of limbs. Ergot alkaloids are also psychoactive. During the Middle Ages historial records indicate periodic epidemics of ergot poisoning.

Lysergic acid is a well known component especially as its amide derviative - lysergic acid diethyl amide (LSD) - to be considered in the next section on CNS drugs. Today ergotamine can be used in the treatment of chronic migraine headache although its side effects do not make it an appropriate prophylactic. It is usually compounded with caffeine and administered orally, by inhalation or suppository. The caffeine increases oral and rectal absorption of the ergotamine. Oral dose is 2 mg at onset and 2 mg at 30 minute intervals up to 6 mg with no more than 10 mg administered per week.



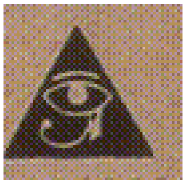
ergotamine (eserine)

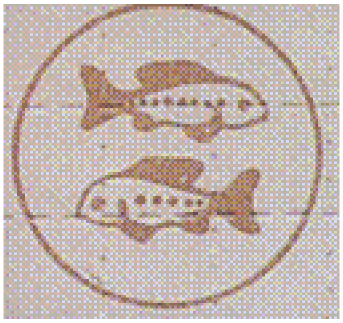
(Ergate®, Ergostat®, Exmigra®, Lingraine®)

Source: ergot from Central European rye fungus

LD_{50}(mice, rats): 62,80 mg/kg (i.v.)

Uses: treatment of migraine



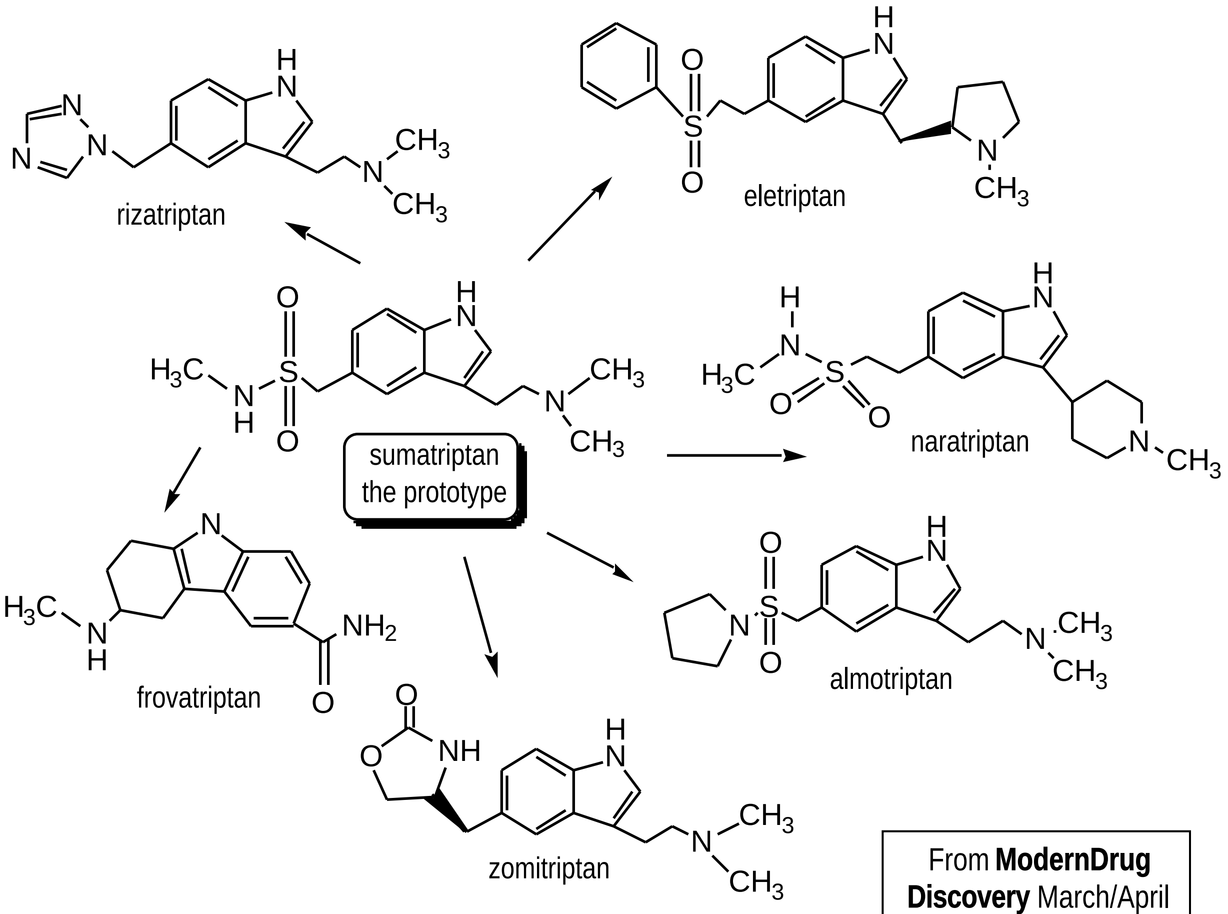


Pictures of blotter papers containing LSD.

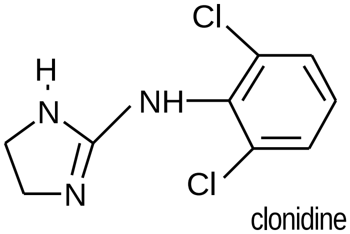
About 23 million Americans, more women than men, suffer from migraine or cluster headaches. With this number of victims you would think that more would be known about the condition and treatment would be more of a science and less of an art. There are several theories concerning the causes of migraine. The most publicized hypotheses attribute the symptoms - tiredness followed by nausea, visual hallucinations and gripping pain - to intracerebral vasoconstriction and dilation of the scalp arteries. CNS serotoninergic neurons are currently in the spotlight as the site of malfunction. At one time aspirin was believed to relieve the clumping of platelets in the blood which might release serotonin. (**Science News** February 17, 1990)

There are some prophylactic treatments which are very individualized. Propanolol (β-blocker) has been used at 80-160 mg/day for up to 12 months. Methylsergide and dihydroergotamine(DHE), relatives of ergotamine, are used but have some serious side effects. Antihistamines such as cyproheptadine (Periactin®) have also been found to have tryptaminergic and anticholinergic activity in the CNS and can be used prophylactically. Calcium channel blockers have also been mentioned in an experimental vein.

The most recent treatment for migraine is a class of compounds known as the "triptans" - sumatriptan and its progeny. These seem to be about 65% effective and show fewer side effects.



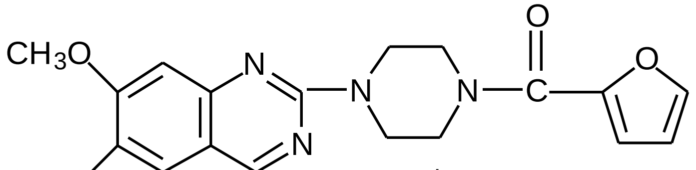
From **ModernDrug Discovery** March/April 1999



clonidine

(Catapres®, Combipres®,Dixarit®)

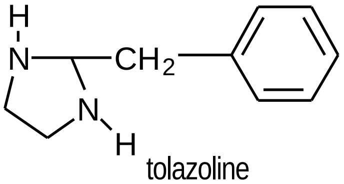
b. Clonidine, an α2-partial agonist/antagonist, and **prazosin**, a quinazoline α-blocker, are used to treat hypertension because of their vasodilatory effects. Clonidine has been administered via a Transdermal patch. Doxazosin mesylate (Cardura®) and terzosin, also quinazolines, are also prescribed.



prazosin

(Minipres®, Minizide®, Hypovase®)

c. Tolazoline is an α-antagonist with cholinergic and antihistamine activity as well. It is used to treat pulmonary hypertension of the newborn and disorders of the peripheral vascular bed such as gangrene and Raynaud's disease. Its antihistaminic actions can cause gastric stimulation. Labetalol also has alpha antagonist activity as well as beta (see next grouping).

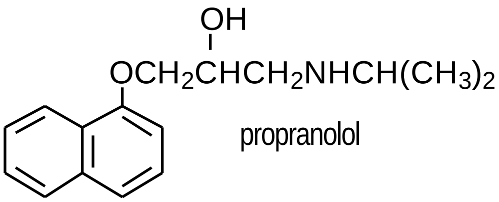


tolazoline

(Priscoline®, Vasodil®, Olitensol®)

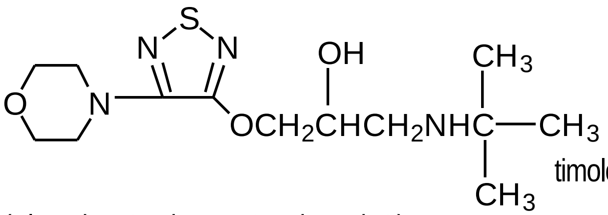
d. Beta-blockers - You might think that if stimulation of β-receptors, especially β_1- receptors, affects the beating of the heart, then blockade of those receptors should lower the heart rate if it is abnormally high. This line of reasoning led to the development of some revolutionary drugs for the treatment of cardiovascular disease, the beta-blockers. **Propanolol**, **timolol**, **nadolol**, and **pinolol** are nonselective while the rest are selective for β_1-receptors when taken in therapeutic doses.

Nonselective beta blockers



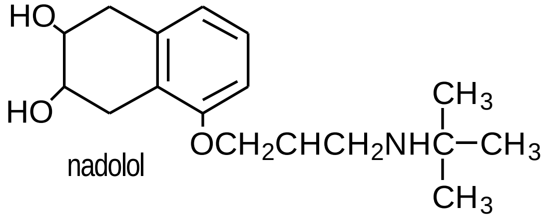
propranolol

(Inderal®, Beta-Neg®, Oposim®, etc.)



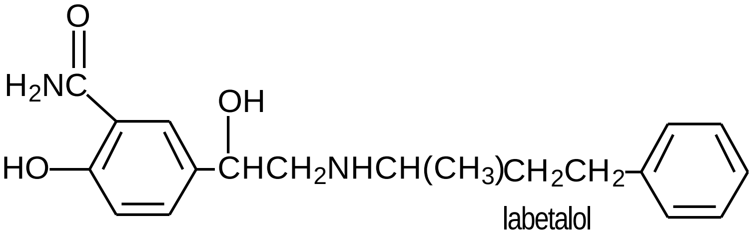
timolol

(Blocadren®, Timacor®, Timoptic®)



nadolol

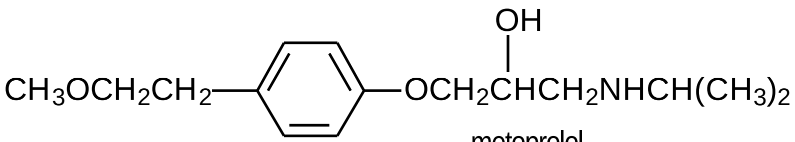
(Corgard®, Solgol®, Corzide®)



labetalol

(Presdate®, Trandate®)

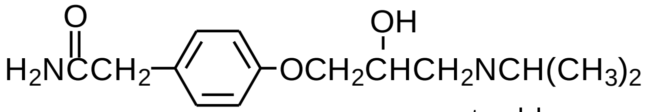
Propanolol, as mentioned before, is sometimes prescribed (though not FDA approved for) the treatment of migraine. It has also been taken by musicians and actors in order to relieve performance jitters. This drug has been specifically prohibited in the Olympic games wherein it is used for fine performance events like marksmanship in the pentathalon.



metoprolol

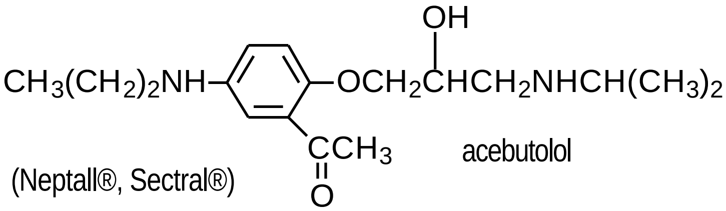
(Betaloc®, Lopressor®, etc)

Selective Beta Blockers



atenolol

(Tenormin®, Tenoretic®)



acebutolol

(Neptall®, Sectral®)

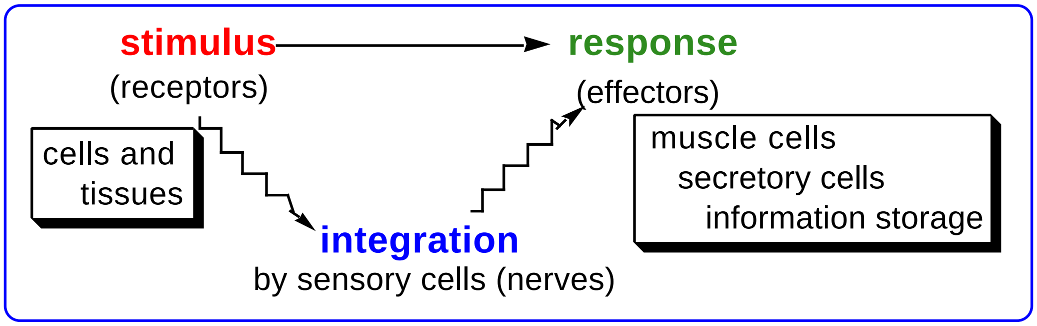
With this we conclude our consideration of the peripheral nervous system and will progress onto the psychoactive enclave of the central nervous system.

DRUGS AND THE NERVOUS SYSTEM

At least one-third of this course is devoted to a discussion of the nervous system and the drugs (toxins) affecting it. While this may seem to be excessive, most of the drugs used therapeutically, and certainly all of those used "recreationally" impinge upon the nervous system. Our coverage will scarcely begin to do justice to the complexity of the topics. Here we are at some of the frontiers of pharmacology and toxicology. So brace yourself.

VII. THE PURPOSE AND NATURE OF THE NERVOUS SYSTEM

As we proceed with this topic it will be necessary to verbally dissect the nervous system into manageable parts in order to understand its functions. However, always keep in mind (no pun intended) that it is a highly organized and integrated network whose main purpose is to keep the organism in a balanced state. This is sometimes referred to as homeostasis. It encompasses billions of individual cells and connections which are responsible for receiving stimuli from the external and internal environments, processing that infinite amount of information, and then responding to those stimuli in a such a manner as to insure the survival of that organism as well as promote its learning processes and foster its propagation. For every stimulus there is a response, be it positive or negative.



A. Components of the Nervous System

1. Nerve cells

The fibrous nerve groupings which can be observed in some parts of the body with the naked eye are called **ganglia**. A ganglion (singular) is

composed of many individual nerve cells or neurons. Neurons can have many shapes and fill various volumes but each one has three essential divisions: the **dendrite**, the **cell body** and the **axon**. In a very simplified description a neuron passes an electrical and chemical message down its length starting with the dendrite, through the cell body and on to the axon. From that point the nerve signal can progress to another neuron's dendrite, or axon to axon, or axon to cell body, etc. The neuron contains many dendrites each capable of picking up and transmitting signals. There is only one axonal ending which can have multiple endings at its terminus. See Figure 20 a. Because neurons can look and act in complex ways, it is convenient to picture the process schematically as in Figure 20 b. The ultimate goal is to convey the message from nerve to nerve, from stimulus to receiving cell/organ/tissue.

ganglion
ganglia

There is a space that exists between neurons known as the synapse. The transmission of the nerve signal across this synaptic cleft is a chemical phenomenon. Molecules generically referred to as neurotransmitters are produced in the neuron and released from the axonal membrane into the synapse. They diffuse to the dendrites of the next nerve cell and combine with receptors. This combination of neurotransmitter (agonist) and receptor produces a response which results in the propagation of the nerve signal down the next neuron. We will discuss this activity in much more detail shortly.

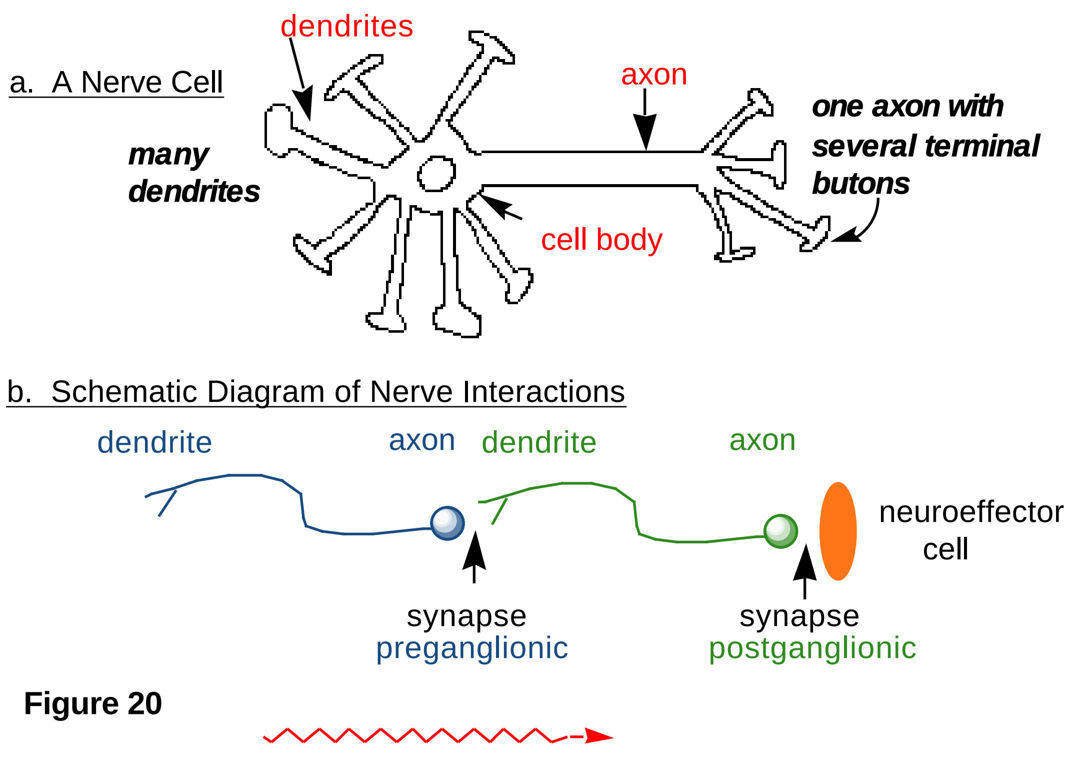
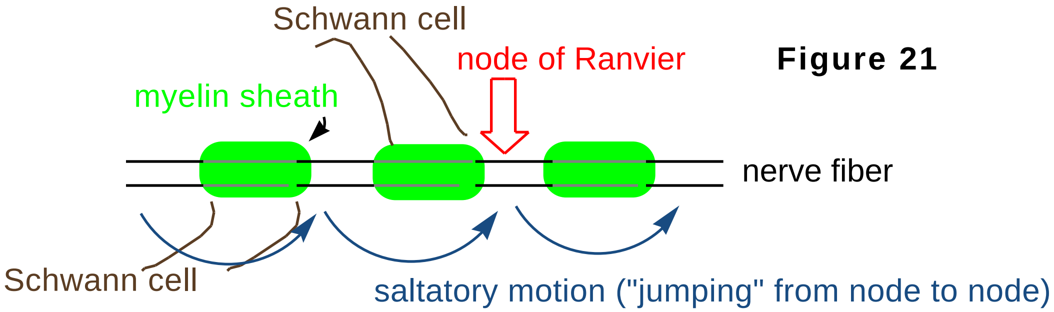


Figure 20

General Direction of Action Potential Generation

Neurons are like most other cells. They have nuclei, mitochondria, endoplasmic reticula, other organelles and membranes - lots of membranes. Some nerve groupings appear grey while others are white. The white matter is a covering for the nerve fiber known as the **myelin sheath**. Myelin is a sphingolipid produced by oligodendrocytes (Schwann cells). It acts as a special type of insulation which allows for a much more rapid transmission of the nerve signal. The myelin cover is interrupted periodically. This "bare" area is called a **node of Ranvier**. It is very important to rapid nerve conduction. See Figure 21.

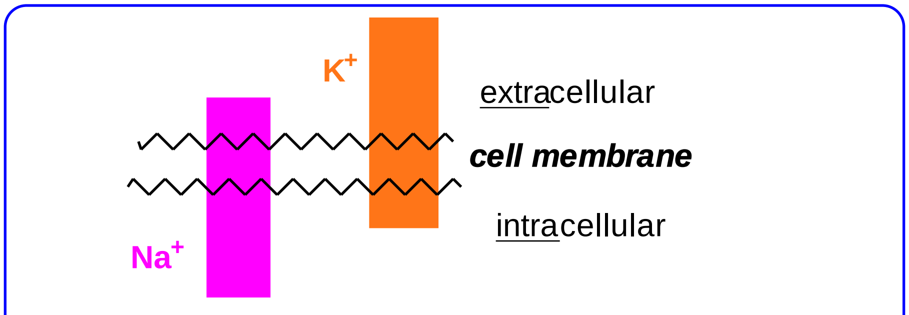
Figure 21



The Myelin Sheath

2. Generation of the nerve signal within a neuron

The propagation of a nerve signal is dependent upon specific membrane phenomena. Remember that the lipid bilayer is a semipermeable barrier which excludes polar materials. Transport of lipophobic species must be either via facilitated diffusion or active transport. Nerve cells use both of these methods to generate an electrical signal. Located in the bilayer are millions of ion pumps, most notably **sodium-potassium ATPase** pumps. These protein complexes exchange sodium and potassium ions so that there will exist an imbalance in the concentrations on either side of the nerve cell membrane. As these positive ions are pumped, negative ions must accompany them. Chloride channels allow the flow of counterbalancing ions. Some positive ions are associated with proteins. These macromolecules cannot leave the cell because of their size. The net result of all of the ion movement is that a potential difference will exist across the lipid bilayer with the inner layer being more electronegative than the outer layer. The average voltage difference is -70 mv inside relative to the outside with variations existing in different nerve tissue.



Ion Concentration Imbalance Across the Cell Membrane

The next event in the generation of a nerve signal involves the opening of sodium channels within the membrane. What will happen considering the imbalance of sodium ions? Of course, they will rush into the cell! The sodium channel opening is following after a brief interval by the opening of the potassium channel. K^+ ions will rush to escape the cell. The potential will change rapidly during these channel openings and what has happened? A localized electical current has been generated called the action potential (AP). When the ions have finished their flow and their channels have closed, the sodium-potassium pump will work to reestablish the ion concentration imbalance. Now, envision the entire length of the neuron covered with this system of pumps and channels. Add to this picture a series of consecutive channel openings. The AP(electrical current) is propagated down the length of the neuron (Figure 22).

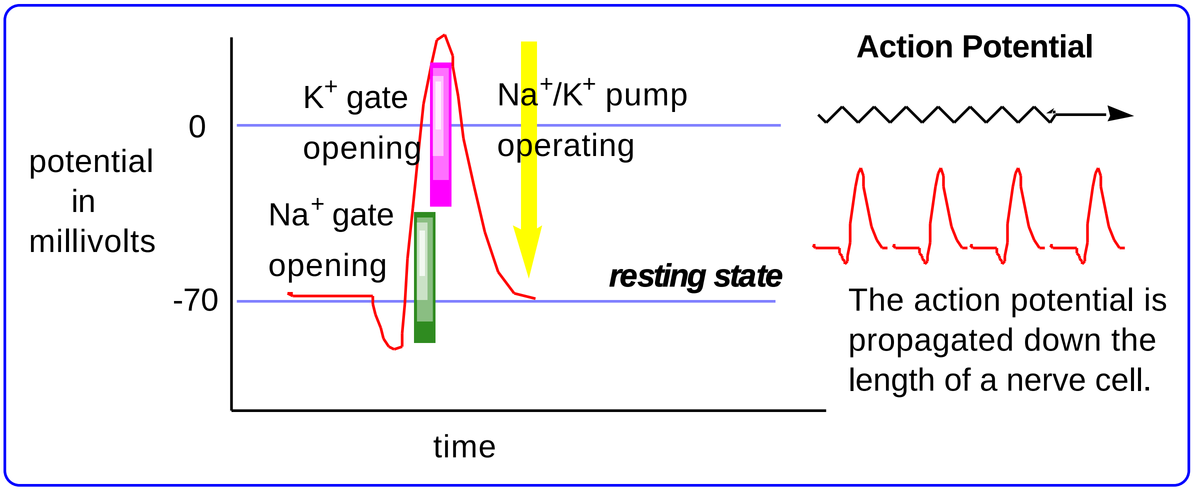


Figure 22
Generation of an Action Potential

It should be noted that the myelin sheath helps to propagate the AP even faster down the neuron. The signal will "jump" from one node of Ranvier to the next (**saltatory motion**). The distribution of sodium and potassium channels is uneven in the myelinated regions, appropriate to the node and covered areas of the nerve. Should the myelin become stripped from a normally myelinated cell, then the electrical signal cannot pass in an "organized" fashion.

Reading: "The Neuron" **Scientific American** September 1979 .

Sodium and potassium are not the only ions which can participate in pumps and channels. Calcium is also pumped, channeled, exhanged,and stored. See Figure 23. Calcium concentration within the cell cytoplasm is very low. This allows the calcium to play a pivotal role in cellular activity. The cytoplasmic protein calmodulin binds and stores calcium ion. Various intracellular structures and organelles such as the mitochondria and sarcoplasmic reticulum also store calcium. Calcium is vital to such functions as the release of neurotransmitters from nerve cells. There are at least seven known modes of biochemical action for this ion, one of the most important of which involves stimulation of cardiac muscle protein (actin-myosin). Certain types of angina (heart pain) are believed to be caused by abnormal stimulation of cardiac arteries and muscle (coronary spasm) A relatively new class of drugs, known as the calcium channel blockers, has brought relief from pain and arrhythmias (irregular heart beats).

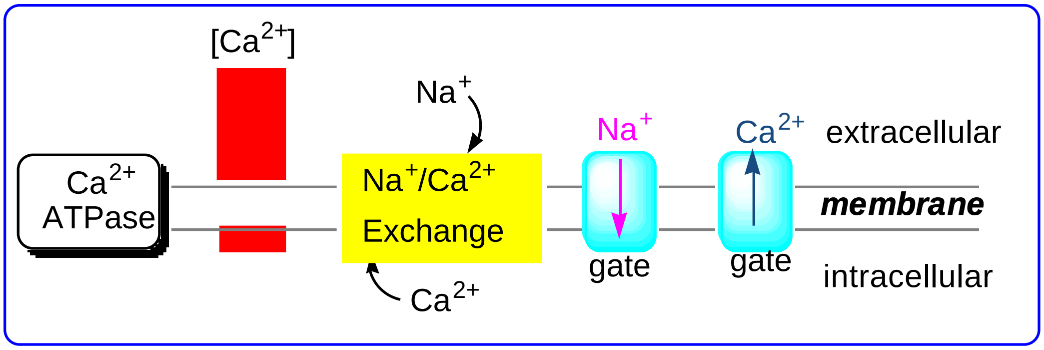
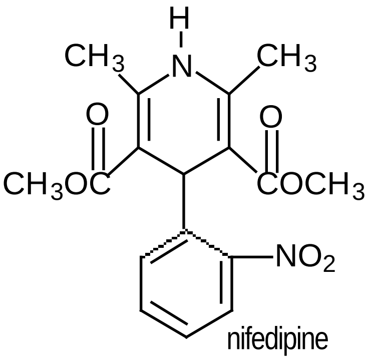


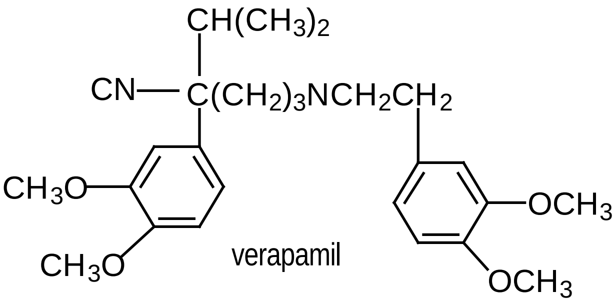
Figure 23 Calcium Exchange in the Cell



nifedipine

Adalat®, Procardia®

initial dose - 10 mg



verapamil

Calan®, Isotopin®, Cordilox®,Vasolan®

initial dose - 5-10 mg

LD_{50} (mice, rats): 16, 8 mg/kg(i.v.)

B. Factors affecting the generation of the action potential

1. Disease - Multiple Sclerosis (MS)

What would happen if the myelin sheath were destroyed and a hard, non-functional cover was substituted at irregular intervals? Nerve conduction would slow, become irratic and, depending upon where the destruction was located, various symptoms such as paralysis and problems with coordination would occur. There are several conditions which can lead to myelin degeneration but one of the most studied is multiple sclerosis, so named because of the matrix of multiple sclerotic (scar-like) plaques of interlocked astrocyte cells which replaces the myelin sheath. Lymphocytes and macrophages as well as antibodies of the immune system surround this insult. Although these components of the immune system are present, there is a strong genetic link for MS within families. Perhaps there are susceptibility genes which make the MS victim a better viral host than someone without the genetic predisposition.

The symptoms of MS include muscle weakness, lack of coordination, difficulty in vision, spasticity, vertigo, facial numbness, tremor, and emotional lability. The course of the condition is unpredictable and may be acute or chronic. The acute form, which is more rare, usually has its onset in middle age and can prove fatal within a year. The more usual course for MS is to strike young adults, 20 to 40 years of age, showing a cyclical worsening of symptoms for more than 25 years, rarely being fatal. MS strikes about 250,000 people per year in the United States(1 in 2000), mostly female. Its occurrence is higher in Western cultures in temperature climates; it is almost unknown in the Middle East. During the Iran hostage crisis of the Carter administration one of the hostages, Richard Queen, was released after he exhibited the symptoms described above. It took medical consultation from Western-educated physicians to diagnose MS. Stress can exacerbate the condition and surely Mr. Queen was under unusual stress.

Various treatments have been tried for the symptoms of MS. Psychological counseling, physical therapy, and treatments with ACTH (adrenocorticotropic hormone) and prednisone for inflammation are standard procedures. In 1983 some MS volunteers were placed in a hyperbaric oxygen chamber in an attempt to suppress the immune system while another group was treated with the immune suppressor drug cyclophosphamide. The results, reported in 1985, showed that the former group showed no improvement over a control group and the results from the latter experiment were questionable since a double-blind study could not be run. Cyclophosphamide causes baldness (alopeica) and those receiving the drug, as well as those receiving the placebo (by default), knew what they were being given. The idea of a **double-blind study** is to make all of the conditions of the experiment the same, with the health care providers,

doctors, nurses, technicians, and the patient not knowing who is receiving what - drug or **placebo** (a nonfunctional pill).

Several other diseases exist which can destroy the myelin sheath. Many are a result of viral infections or the body's immune response to a bacterial infection. Guillain-Barre syndrome causes numbness and paralysis starting at the extremities radiating into the trunk of the body where it can cause complete respiratory collapse. Guillain-Barre has occurred in senior citizens who have been given flu vaccinations. The virus used in the vaccination may be attenuated (diminished in virility but not "killed") and still be capable of causing an infection.

Subacute sclerosing pancephalitis can occur years after a measles vaccination. This is usually a fatal condition.

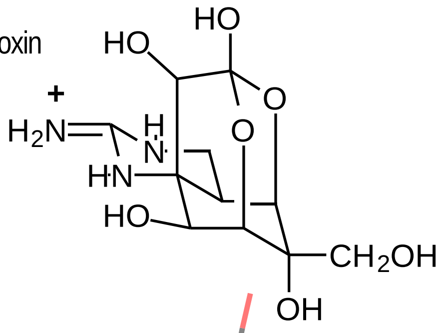
2. Toxins

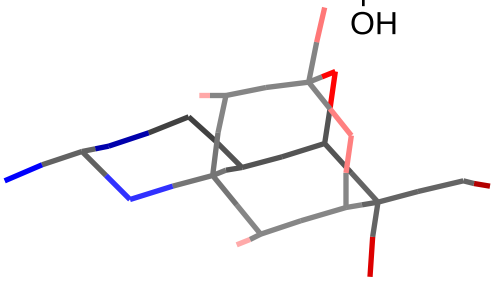
There are few drugs designed to interfere with the action potential because of the inherent non-selectivity of the treatment. Some local anesthetics such as benzocaine and cocaine can block sodium channels and so prevent the propagation of a pain signal. These drugs also have other effects which will be covered shortly. It is toxins which are of interest here since such shorting out of the AP would produce life-threatening results. I will categorize these toxins according to their site of action.

Please notice that from now on the information given for each drug or poison includes its structure, source, the symptoms it produces, its uses (other than that of drug or poison), LD_{50}, and the antidote (if known).

The first group are sodium channel blockers which interfere with the propagation of the action potential.

tetrodotoxin



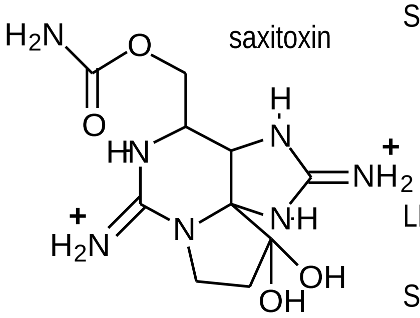


Sources: flora inhabiting puffer fish (roe, liver, skin), some newts, octopi and frogs, gobys, the California salamander

LD_{50} (mice): 10 mg/kg (i.p.)

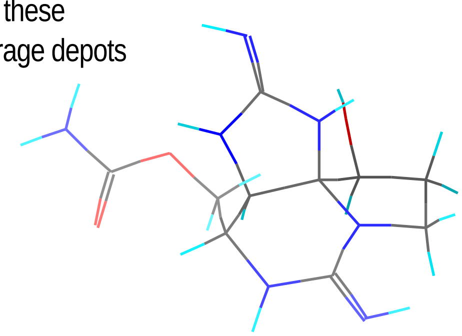
Symptoms: spreading paralysis
Antidote: none (60% fatality)

Uses: Puffer fish is a Japanese delicacy. A chef must be licensed to prepare puffer fish. Some thrill seekers will prepare it improperly to see how far they can go.



saxitoxin

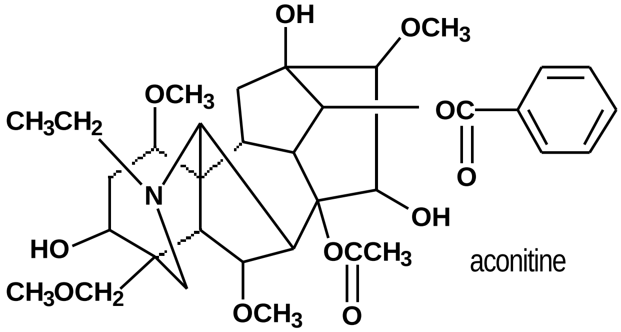
Sources: dinoflagellates *Ginoyaulax catella* - the "red tide"- shellfish feed upon these mircroorganisms and become storage depots for the toxin



LD_{50}(mice, rats): 531 mg/kg (oral)

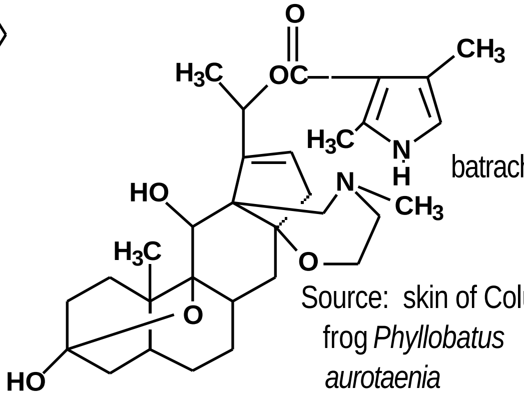
Symptoms: paralysis
Antidote: none

The next group acts by keeping the sodium channels open. This prevents the buildup of the ion imbalance so necessary for the AP generation. This type of activity, that of preventing the -70 mv potential difference across the membrane, is referred to as persistent depolarization.



aconitine

Sources: *Aconitum napellus, Ranunculaceae*
LD_{50} (mice): 1 mg/kg (oral)
Symptoms: heart arrhythmia
Use: arrow poison

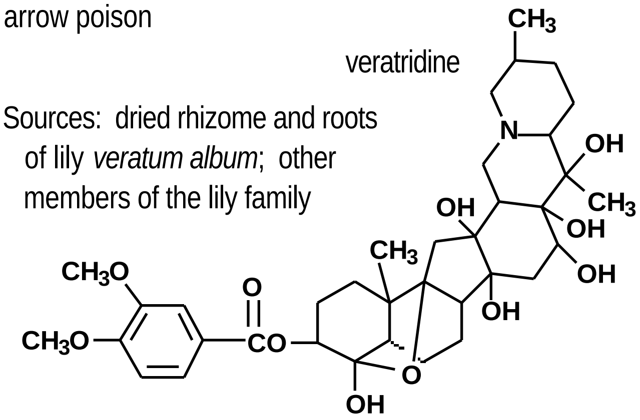


batrachotoxin

Source: skin of Columbian frog *Phyllobatus aurotaenia*
LD_{50}(mice): 1 mg/kg (i.v.)
Use: arrow poison

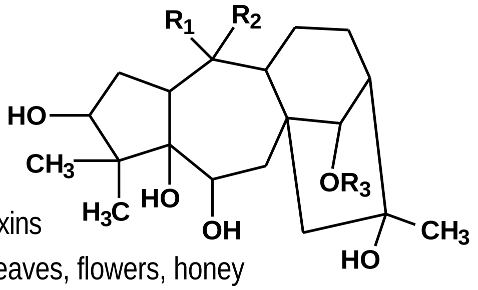
veratridine

Sources: dried rhizome and roots of lily *veratum album*; other members of the lily family



LD_{50}(mice): 1.35 mg/kg (i.p.)
Symptoms: general paralysis
Uses: as a veterinary emetic (mixture with other alkaloids)

There have been some interesting conjectures presented by certain ethnobotanists that associate the use of sodium channel blockers like tetratotoxin with the practice of occult magic such as that found in voodoo rituals in the Caribbean. Wade Davis gained notoreity in the late 1980s with his articles and book **The Serpent and the Rainbow** in which he stated that practitioners of this ancient belief drugged their victims with a brew which included tetratotoxin. This put the unfortunate party into a trance-like state close to death. The person would be buried and then exhumed later to be further drugged and forced into servile labor as a "zombie" (the living dead).

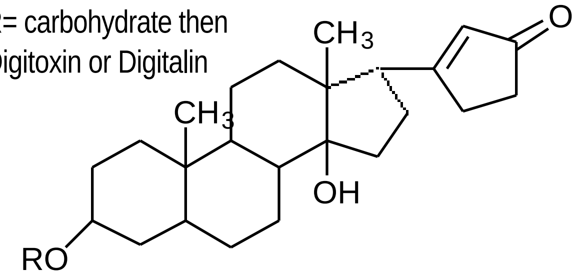


grayanotoxins
Sources: leaves, flowers, honey of *Rhododendron*

R_1	R_2	R_3	LD_{50} (mice) (i.p.)
OH	CH_3	$COCH_3$	1.30 mg/kg
$=CH_2$	$=CH_2$	H	26.2
OH	CH_3	H	0.84

Digitoxin and its derivatives bind to the sodium-potassium pump and prevent it from exchanging sodium and potassium ions. When given in small quantities the result, especially in cardiac tissue, is that calcium ion content will be upset and calcium will be liberated from its stores. The newly available calcium can then interact with cardiac muscle protein to cause contraction. This is a therapeutic effect for those suffering from insufficient heart-pumping action (congestive heart failure or dropsy). The heart is made to contract efficiently and forcefully (inotropic effect). However, you can see that there may be a fine line between a necessary contraction and one which

Digitoxigenin R=H
If R= carbohydrate then Digitoxin or Digitalin



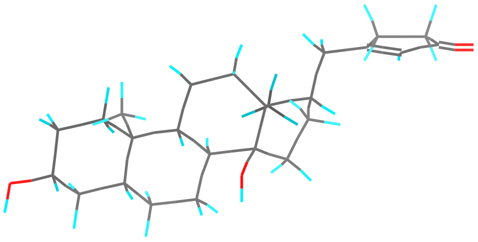
The group of active compounds are known as the cardiac glycosides.

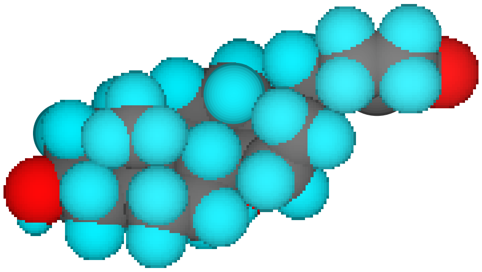
Sources: foxglove, purple foxglove, fairy gloves

LD_{50}(guinea pigs, cats): 60.0, 0.18 mg;kg
The therapeutic dose is close to the toxic dose.

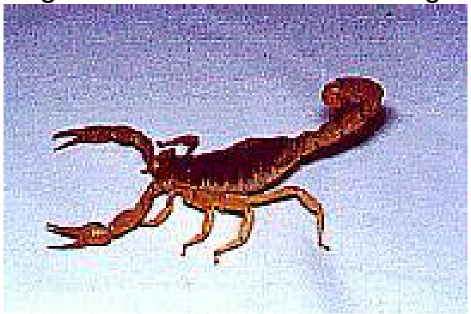
Symptoms: anorexia, nausea, salivation, vomiting, headache, drowsiness, disorientation, delirium, hallucinations; can be fatal.

Uses: cardiac stimulant (Crystodigin®, Lanoxin®, Digibind®)





might send the heart into irregular beating and so lead to death.



Scorpion toxins are polypeptides containing 30-78 amino acids. They have their actions on sodium channels as well as at other locations. Charybodotoxin, from the scorpion ***Suries quinquestriatus*** is a 37 amino acid peptide which has potassium channel blocking activity. The really toxic scorpions are found mainly in Mexico and North Africa. Fatalities usually occur in very young children or the elderly, especially if they are weakened by illness. It has been reported, for example, that three-fourths of the scorpion sting fatalities in Mexico occur in children less than 4 years old.

Certain species of **sea anemones** produce basic polypeptide toxins of molecular weights ranging from 2500 to 5000 daltons. They cause persistant activation of sodium channels and lead to paralysis. This is very helpful to the sea anemone is harvesting its prey.

Predatory cone snails produce a variety of polypeptide toxins (**conotoxins**) in their venoms which are rich in disulfide bonds made by the amino acid cystine. The activities of these toxins include Na^+ and K^+ channel blocking. Intoxication by the proteinaceous toxins mentioned above will produce symptoms of severe local pain (due to other compounds in the venom), sweating, nausea, cramps, hyperglycemia, and respiratory distress.

It is well to note here the difference between a venom and a toxin. A **venom** is a mixture of compounds which each have its own biological action and the chemicals often act in concert to enchance the toxic effects. A **toxin** is one of the components of a venom, a distinct chemical entity, frequently a protein. It is isolatable and it usually exerts a single biochemical action.

See the ASIDE on dendrobid frogs which illustrates some of the toxins which are found in these amphibians of the South American jungles.

C. Divisions of the Nervous System

There is really only one, whole, integrated nervous system. However, for the purposes of understanding the effects of drugs and poisons on the system, we will subdivide it anatomically and functionally. Afferent nerves

are those which carry signals to the brain; efferent nerves carry the signal away. Most of the time it will seem as if we are discussing the efferent system, but what is learned can also apply to the afferent.

Anatomically the nervous system is subdivided into the Central Nervous System (CNS), that which is encased in the cranial bone tissue and surrounded by the bone and cartilage, that is, the brain and spinal column, respectively. The function of the CNS is to control and integrate afferent and efferent signals. The network of neurons in the soft tissues of the bodies, impinging on organs and musculature is the Peripheral Nervous System (PNS). We will discuss the CNS in more detail later. For now, let us consider the PNS.

The PNS starts with 12 pairs of cranial nerves extending from the upper portion of the spine and 31 pairs of nerves coming out of the mid to lower spinal column. See Figure 24.

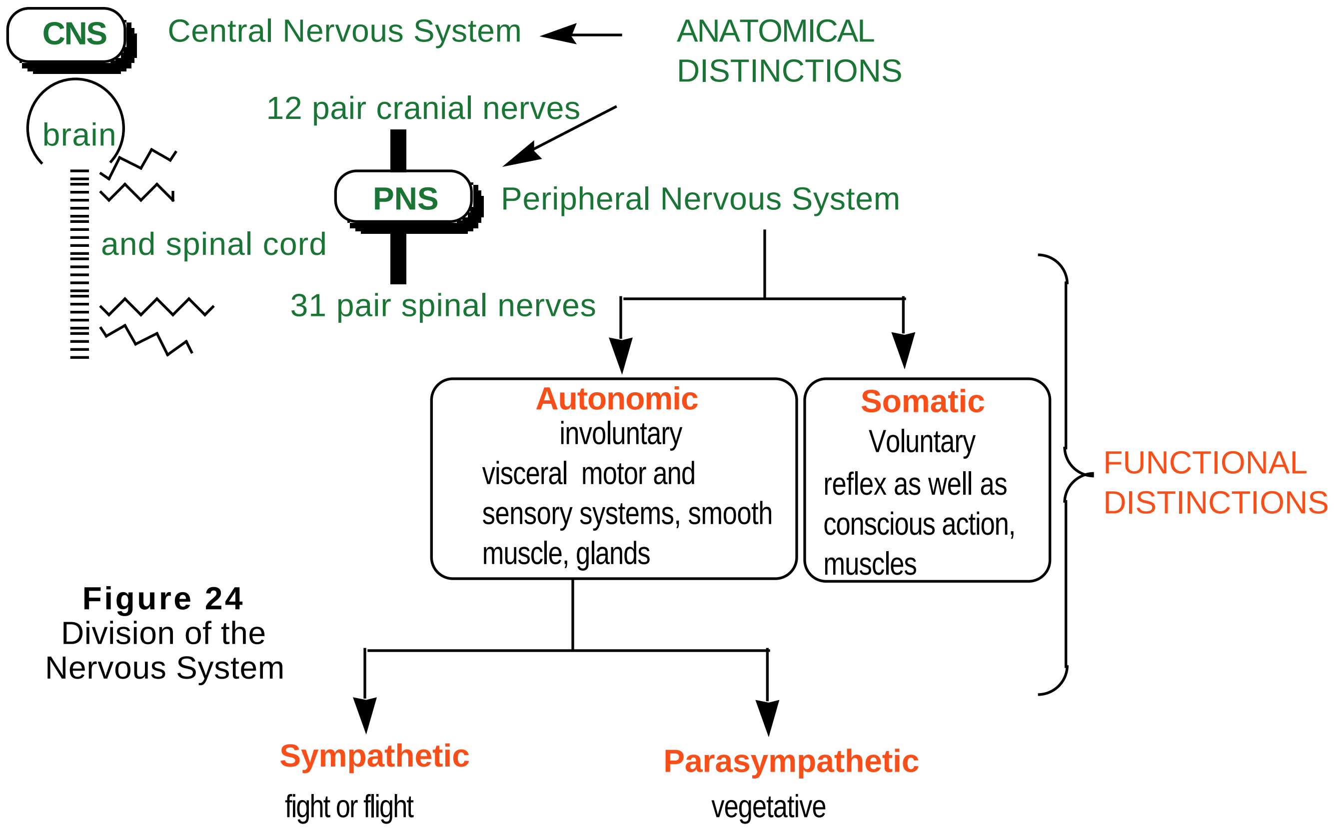


Figure 24
Division of the Nervous System

The PNS is further divided into functional sections known as the autonomic and somatic systems. The autonomic nervous system is also called the involuntary system. It regulates, without conscious effort, the visceral motor and sensory organs and muscles, as well as other smooth muscle and glands. The somatic is the voluntary nervous system which

controls reflex as well as conscious muscle action. Most of our ensuing discussion will involve the autonomic nervous system. It, in turn, is divided into two biomolecular/functional parts: the sympathetic and parasympathetic systems. Every organ in the body with the exception of the adrenal medulla, which is affected only by the parasympathetic, is ennervated by both of these systems.

The sympathetic part of the nervous system(SNS) regulates our response to threat or stress. It is sometimes called the "fight or flight" response. The nerves coming from the spine(preganglionic fibers) in this system are short while those leading to the organs(postganglionic fibers) are longer. There are many postganglionic fibers for every preganglionic one. So it is a very diffuse system and can elicit a broad response.

In contrast, the parasympathetic nervous system(PNS) is our vegetative system, that is, it controls automatic processes such as digestion. Its main purposes are energy conservation and restoration. The preganglionic fibers in the PNS are long and the postganglionic are short. The ratio of pre- to post- is low which means the PNS is not as diffuse as the SNS.

The SNS and PNS counteract and thereby balance each other in the regulation of the autonomic functions of the body. Should one system become inoperable the other will dominate. Table 5 lists the responses of various tissue and organs to SNS and PNS stimulation. Figure 24 summarizes the details of the autonomic nervous system.

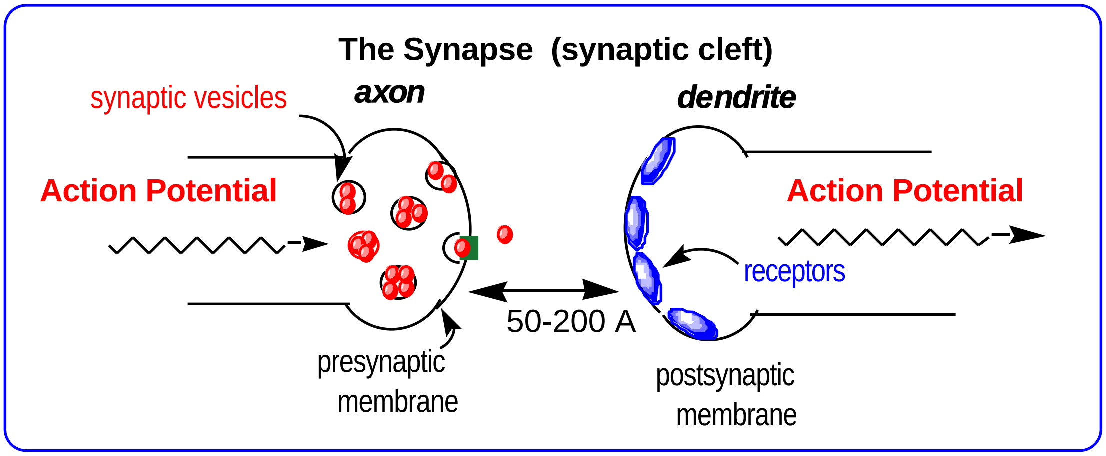
TABLE 5: The Autonomic Nervous System

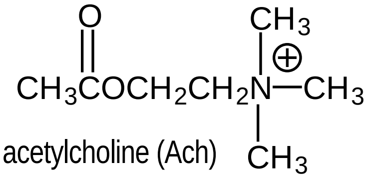
SNS Effects	Organ or Tissue	PNS Effects
Dilated	EYES	Contracted
Increased	HEART RATE	Decreased
Increased	BLOOD PRESSURE	Decreased
Elevated	BLOOD GLUCOSE	Normal
Dilated	BRONCHIOLES	Constricted
Increased	RED BLOOD CELL PRODUCTION	Normal
Stimulated	SWEAT GLANDS	Normal
Thick Secretion	SALIVA	Watery Secretion
Inhibited	PERISTALSIS	Stimulated
Inhibited	DIGESTION	Normal

D. Neurotransmitters

The sympathetic and parasympathetic nervous systems can be further categorized by the types of molecules which act as messengers from one

neuron to another - the neurotransmitters. These biomolecules are synthesized in the presynaptic neuron and stored in organelles known as presynaptic vesicles. When the action potential reaches the area of the axonal membrane, the vesicles with their stored molecules, fuse with the presynaptic membrane and empty their contents into the synapse. The neurotransmitters then diffuse through the 50-200 Angstrom synaptic cleft until they reach an appropriate receptor on the postsynaptic membrane. The time for this excursion is 0.1-0.2 milliseconds. The neurotransmitter-receptor complex formation will trigger the generation of an action potential in the next neuron.



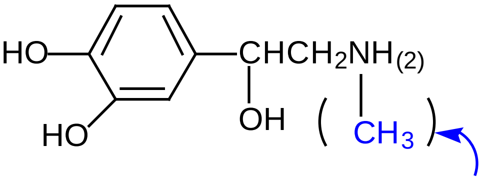


acetylcholine (Ach)

1. Acetylcholine (Ach)

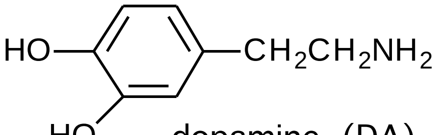
The molecule acetylcholine is a neurotransmitter in both the PNS and SNS. It also plays an essential role in several areas in the CNS. Synapses which have Ach as their principal neurotransmitter are known as cholinergic systems.

2. Norepinephrine (NE)



norepinephrine (NE) epinephrine - E

Norephinephrine is found exclusively in the sympathetic nervous system in the postganglionic connections. It also is a key neurotransmitter in many areas of the CNS. Such neurons are called adrenergic for the other term used for norepinephrine, noradrenalin. Adrenalin (epinephrine) is a hormone produced by the adrenal medulla and it can also ennervate the sympathetic system.



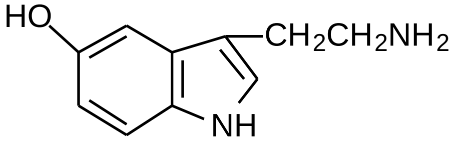
dopamine (DA)

3. Dopamine (DA)

Dopamine is both a precursor to norepinephrine and a neurotransmitter in its own right. We will discuss dopamine mainly with respect to its action in the CNS. DA systems are called dopaminergic.

4. Serotonin (5-HT for 5-hydroxytryptamine)

Serotonin also has most of its activity in the CNS. Serotonergic systems are important in sleep and the integration of incoming stimuli.



serotonin (5-hydroxytryptamine - 5-HT)

5. Nitric Oxide - NO (Molecule of the Year 1993)

The latest entrant in the neurotransmitter lineup is NO, nitric oxide, the first neurotransmitter discovered in the gas phase. For decades physicians and pharmacologists have been aware of the ability of nitrates to dilate blood vessels. Compounds like nitroglycerin are key tools in the control of angina pectoris, constriction of the coronary arteries. Yet no one knew exactly how the nitrates performed their feat until recently. The notariety of Viagra® attests to the interest in products which affect NO systems. More about the synthesis and actions of NO will be described shortly.

E. Biosythesis and degradation of neurotransmitters

The general scheme for the life cycle of a typical neurotransmitter, with the exception of NO, is illustrated in Figure 25.

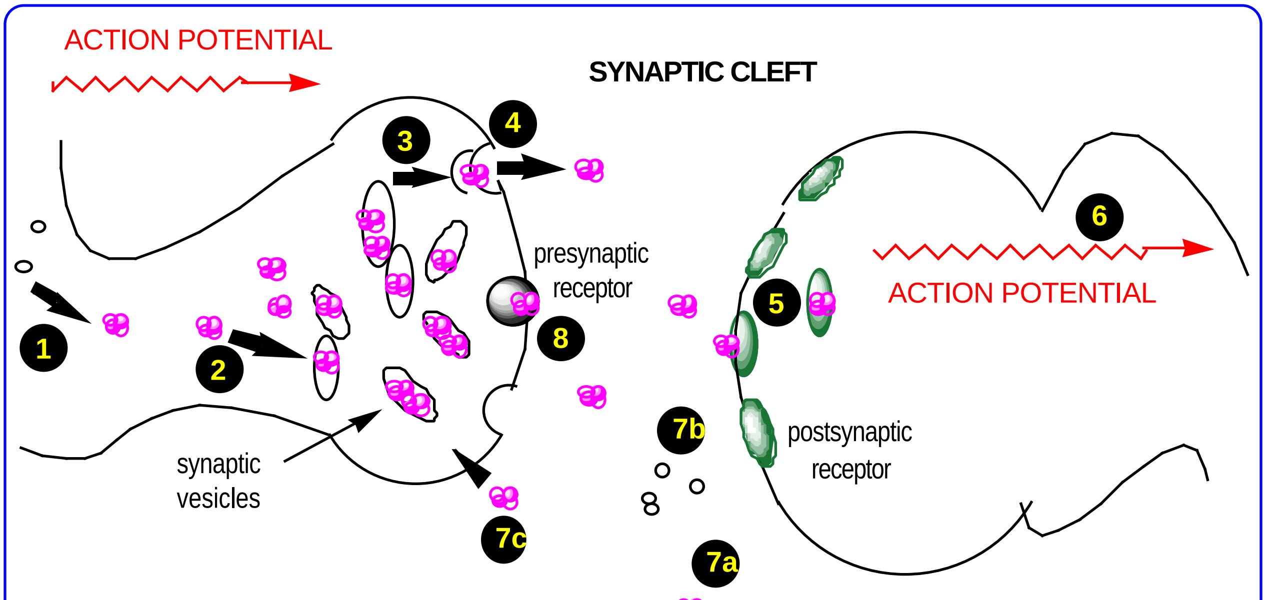
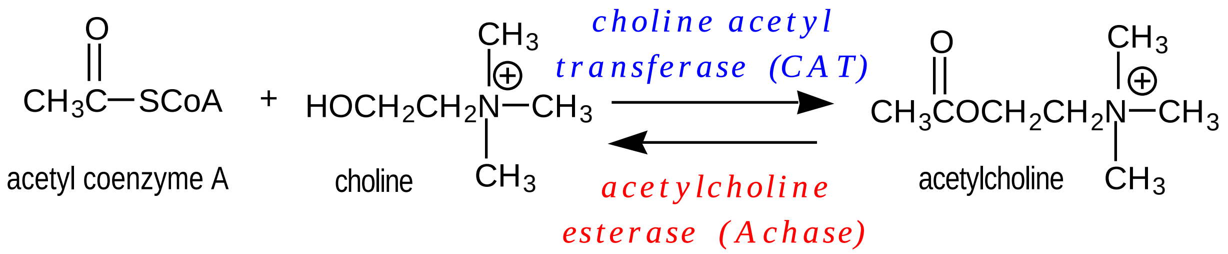


Figure 25
The Life Cycle of a Neurotransmitter

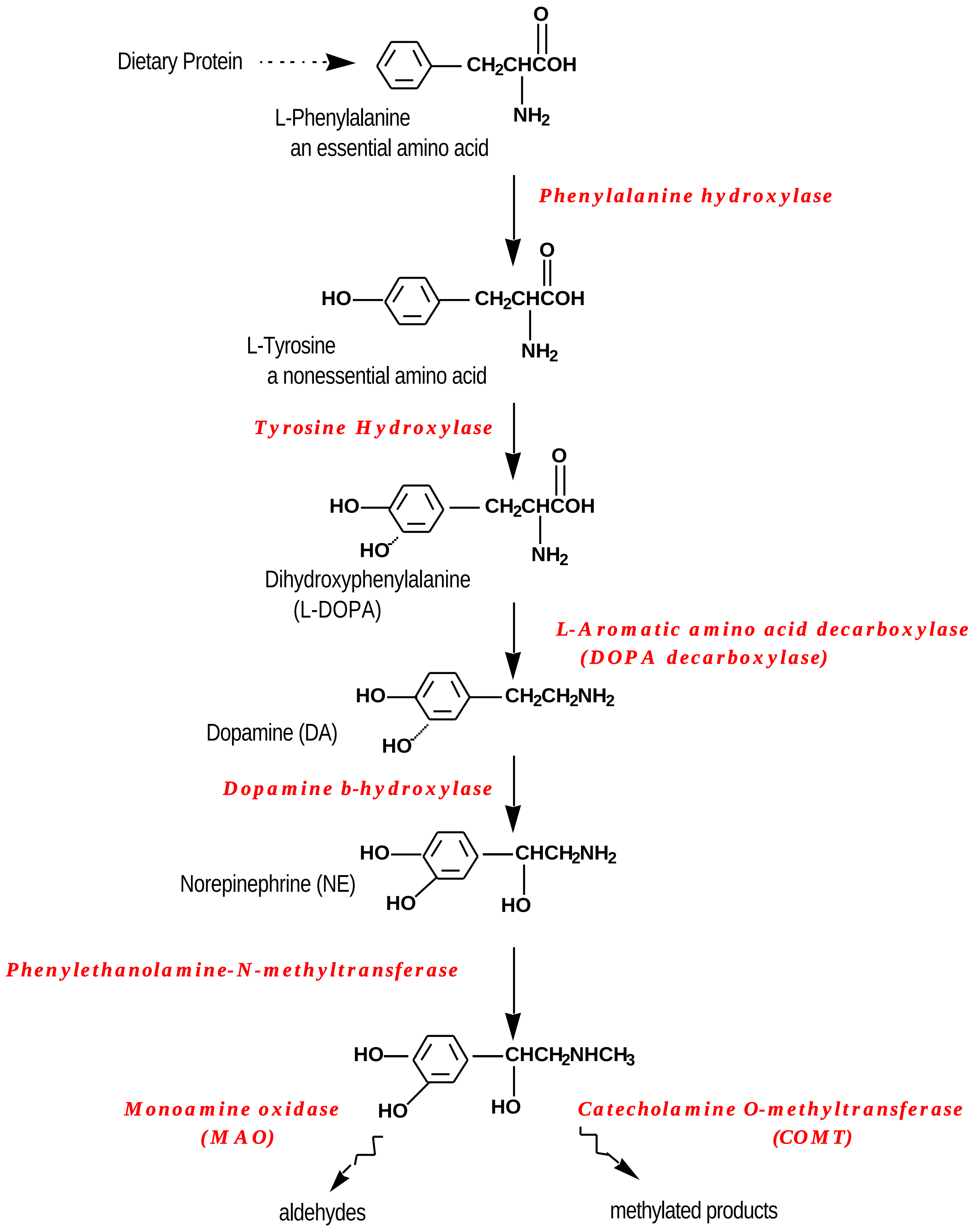
1	biosynthesis of NT	**6**	the NT-receptor complex triggers generation of the AP
2	storage of NTs in synaptic vesicles	**7a**	NT dissociates from receptor and either diffuses away,
3	arrival of AP trigger fusion of vesicle with synaptic membrane and	**7b**	is enzymatically broken down, or
4	release of neurotransmitter	**7c**	taken back up into the presynaptic ending.
5	NT diffuses to postsynaptic membrane where it combines with a receptor.	**8**	The NT may also bind with presynaptic receptors as a regulatory device for NT release.

As was mentioned before, neurotransmitters are synthesized within the neuron either in the cell body or in the synaptic area and stored in presynaptic vesicles.

Acetylcholine is an ester composed of acetate (from acetyl coenzyme A) and choline (either synthesized ***de novo*** or taken in the diet as lecithin). The enzyme choline acetyl transferase catalyzes the reaction to form Ach. The breakdown of Ach back to acetate and choline, which will terminate its activity, is catalyzed by the enzyme acetylcholineesterase (Achase).



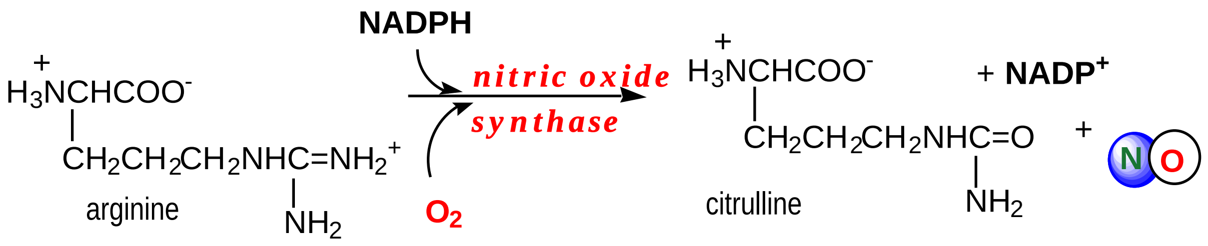
Dopamine, norepinephrine and epinephrine are products of the metabolism of dietary phenylalanine. This is an interesting sequence of reactions in that we will be discussing not only the three neurotransmitters formed but also considering the DOPA precursor and its use in the treatment of Parkinson's Disease. These molecules are also called catecholamines. Catechol is an ortho dihydroxyphenyl derivative. Degradation of the final product in the pathway, epinephrine, can be accomplished by oxidation (monoamine oxidase - MAO)or methylation (catecholamine O-methyl transferase - COMT). The diagram on the next page illustrates the scheme of successive oxidations which produce the various catecholamines.



Dietary Protein
CH2CHCOH
O
NH2
L-Phenylalanine
an essential amino acid
Phenylalanine hydroxylase
HO
CH2CHCOH
O
NH2
L-Tyrosine
a nonessential amino acid
Tyrosine Hydroxylase
HO
HO
CH2CHCOH
O
NH2
Dihydroxyphenylalanine
(L-DOPA)
L-Aromatic amino acid decarboxylase
(DOPA decarboxylase)
HO
HO
CH2CH2NH2
Dopamine (DA)
Dopamine b-hydroxylase
HO
HO
CHCH2NH2
HO
Norepinephrine (NE)
Phenylethanolamine-N-methyltransferase
HO
HO
CHCH2NHCH3
HO
Monoamine oxidase
(MAO)
Catecholamine O-methyltransferase
(COMT)
aldehydes
methylated products

Nitric Oxide is synthesized from arginine through catalysis by the enzyme ***nitric oxide synthase*** (NOS) in the postsynaptic neuron. The paramagnetic, diatomic molecule can activate guanyl cyclase within its host neuron or it can diffuse through the membranes of the neuron to enter the presynaptic neuron and activate the guanyl cyclase second messenger system there. NOS is itself activated by a second messenger system - a Ca^{2+} - calmodulin complex. There is much more to the NO story which will be mentioned later in 377.

This discovery and its subsequent development led to Viagra® as well as the 1998 Nobel Prize in Physiology or Medicine to R. Furchgott, F. Murad, and L.J. Ignarro.



F. Sympathetic and parasympathetic systems - neurotransmitters and receptors

The parasympathetic system is exclusively a cholinergic system. The neurotransmitter acetylcholine is produced at both pre- and post-ganglionic nerve cells. Acetylcholine is the neurotransmitter at the preganglionic neurons of the sympathetic system also, but the postganglionic sympathetic stimulation is by norepinephrine.

Besides the differentiation of cholinergic and adrenergic neurons in these systems, there is also a variation in the protein receptors with which the neurotransmitters complex at the postsynaptic membrane.

In the parasympathetic cholinergic neurons the receptors in the preganglionic synapse are called **nicotinic** receptors (for the compound nicotine which is a xenobiotic agonist for them). The postganglionic receptors may be either nicotinic, if they are on a muscle cell, or **muscarinic** (for the compound muscarine, an agonist), if they are on an organ or other nonmuscle cell. Nicotinic receptors are pentameric proteins exhibiting heterogeneity in the association of their subunits (α to γ). For example, muscle nicotinic receptors have a different subunit composition ($\alpha_2\beta\delta\gamma$ or $\alpha_2\beta\delta\varepsilon$) than those found in the CNS (ab in various combinations). Muscarinic receptors are glycoproteins of molecular weight 80 kdaltons and

come in three types: M_1, which occur in the CNS and autonomic ganglia; M_2 affecting the heart; and M_3 in smooth muscle and secretory cells.

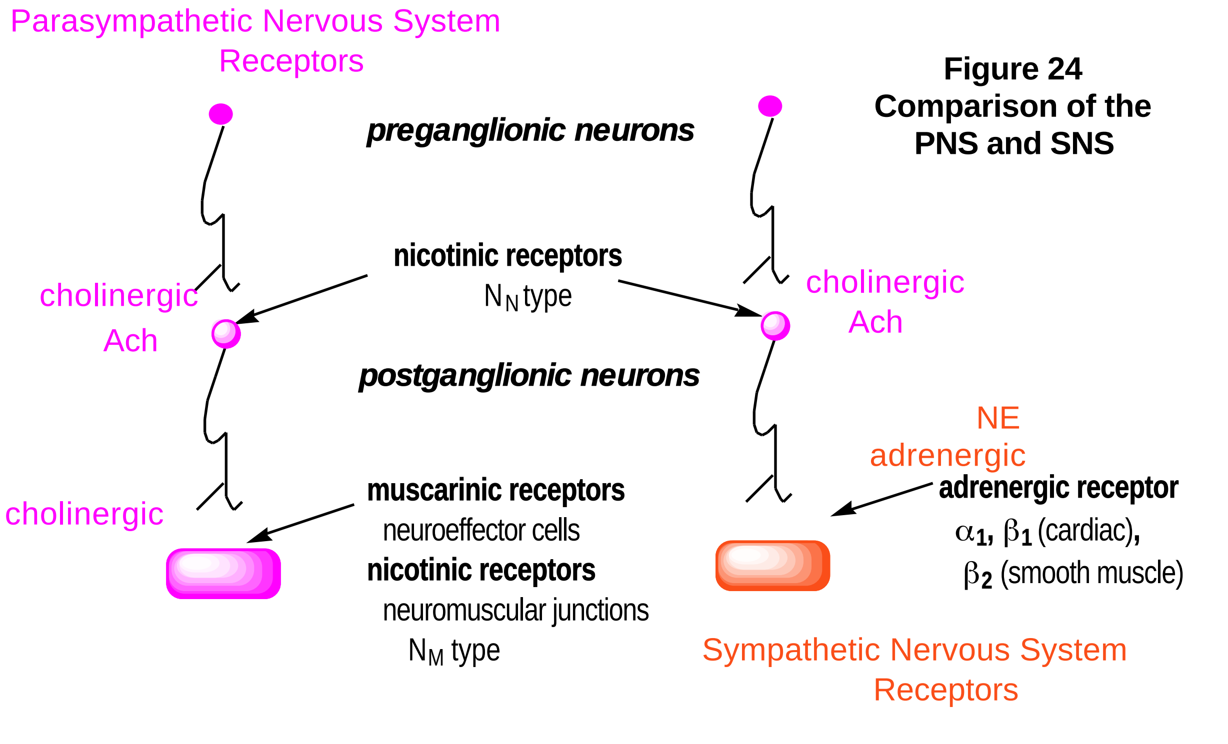


Figure 24
Comparison of the PNS and SNS

In the sympathetic system the preganglionic cholinergic receptors are nicotinic while the postganglionic are adrenergic variations called α and β receptors. These receptors are also subdivided and indicated by subscripts in order to specify the tissues where they are found.

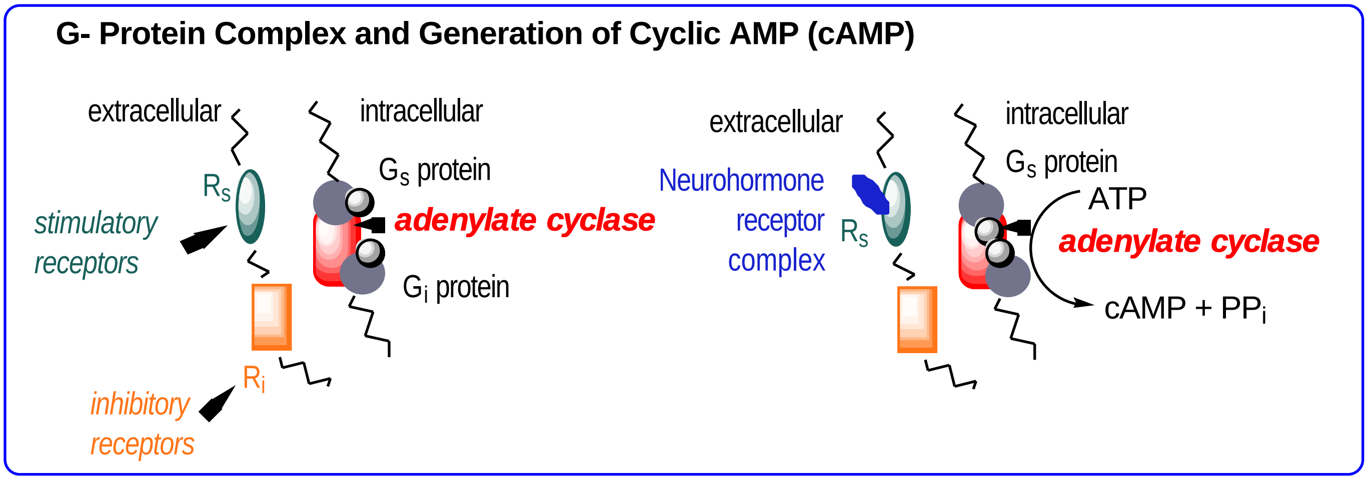
α_1 receptors are postsynaptic while α_2 are presynaptic. Up to this point you may have thought that all receptors are on the postsynaptic membrane. This is not so. Receptors can also be found on presynaptic axonal membranes where their stimulation by a neurotransmitter may act as a control mechanism for the release of more neurotranmitters from the synaptic vesicles. β_1 receptors are usually found on cardiac tissue and on cells where their stimulation can result in lipolysis (breakdown of lipids). β_2 receptors are found on smooth muscle and skeletal muscle. Stimulation of smooth muscle causes it to relax while stimulation of skeletal muscle causes it to contract.

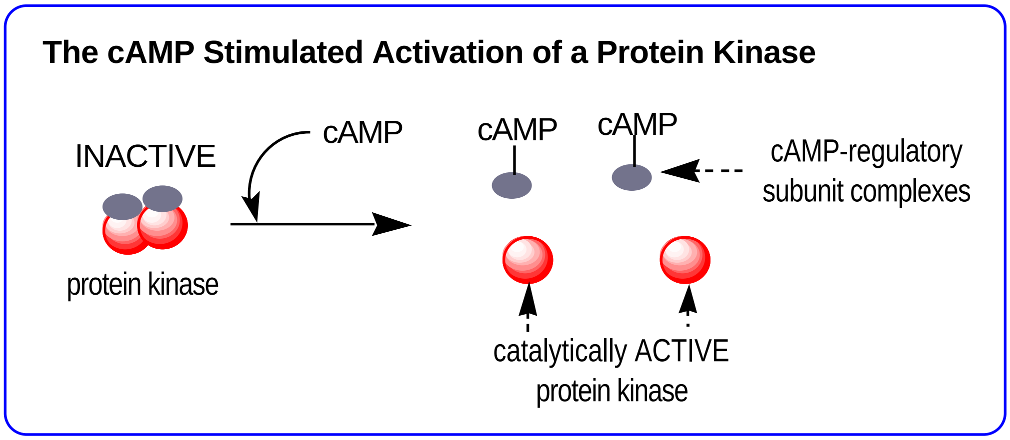
The effects of the agonism of these receptors differs depending upon whether it is a cholinergic or adrenergic stimulation. Cholinergic agonism of nicotinic receptors leads to the opening of ion channels so that the action potential may be directly generated in the neuron. Cholinergic muscarinic stimulation as well as adrenergic agonism leads to the activation of the "second messenger" system - the formation of cAMP (cyclic adenosine monophosphate) or cGMP (cyclic guanosine monophosphate). These

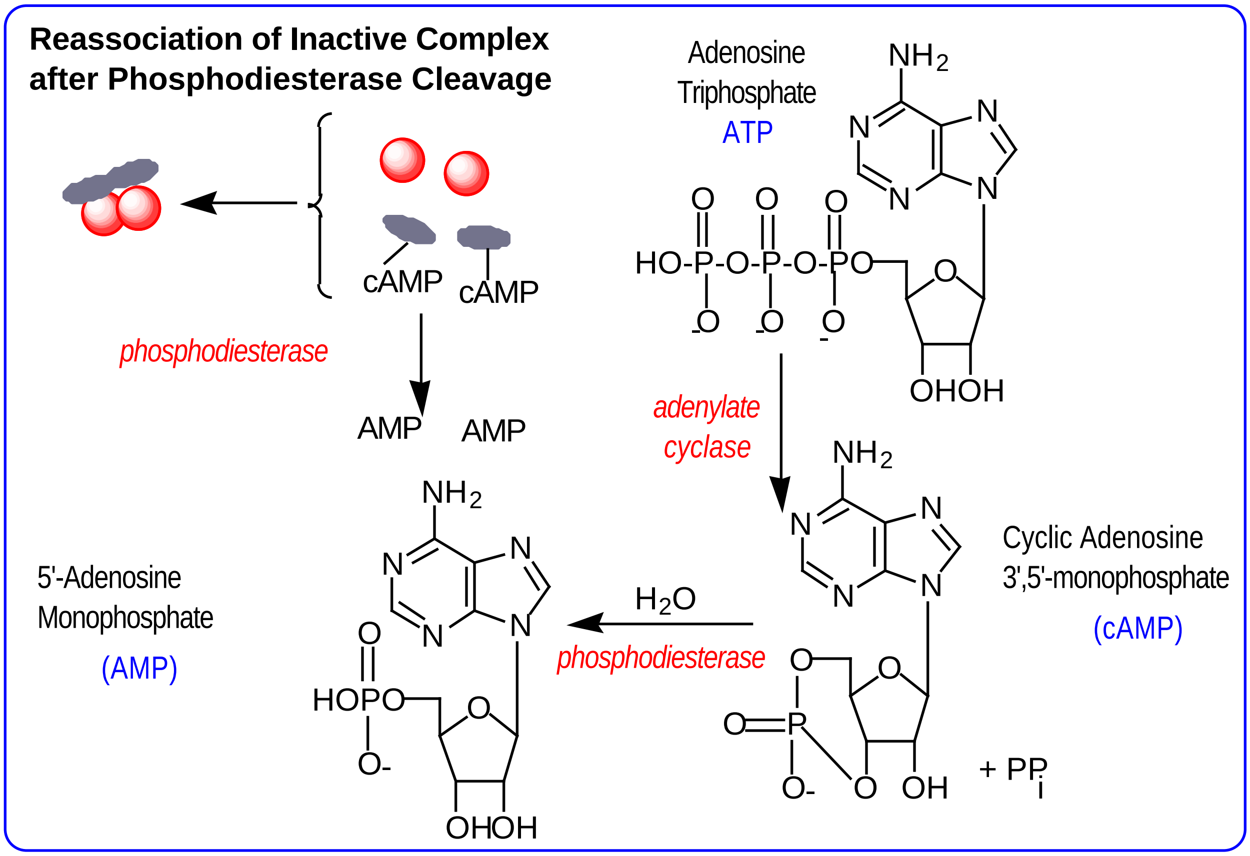
second messenger systems are part of an enzyme-receptor complex referred to as the "superfamily of G-proteins", that is , those which bind GTP/GDP in the elaboration of their mechanisms. This will indirectly cause the generation of the action potential. See Figure 25 for an outline of this second messenger system.

The second messenger system can be affected by a number of xenbiotics both simple molecules and bacterial toxins. See the ASIDES which discuss the effects of methyl xanthines such as caffeine as well as the effects of cholera and pertussis (whooping cough) toxins on cyclic AMP.

Figure 25







Another second messenger system, the inositol triphosphate-diacylglycerol system, can also be activated by cholinergic or adrenergic receptors. It involves calcium movement and will be discussed when and if time permits.

We are discussing the peripheral nervous system at this point. Many of the NTs mentioned now will appear in the section on the Central Nervous System but their modes of neuronal action may be very different.

ASIDE: INVASIONS OF THE SECOND MESSENGER SYSTEM

The Actions of Cholera and Pertussus Toxins

Cholera is a condition caused by a protein exotoxin produced by the bacterium ***vibrio cholerae.*** This protein toxin consists of six subunits: one A subunit and five B subunits. The B subunits are responsible for the binding of the toxin to cAMP-functioning cells in small bowel of the intestines. The A subunit penetrates the cell and has catalytic activity which attaches the ADP portion of naturally occurring NAD (nicotine-adenosine dinucleotide) to the G-protein complex thereby inhibiting its GTPase activity. This deprives the complex of its "off-switch" for cAMP formation. The effect is the uncontrolled

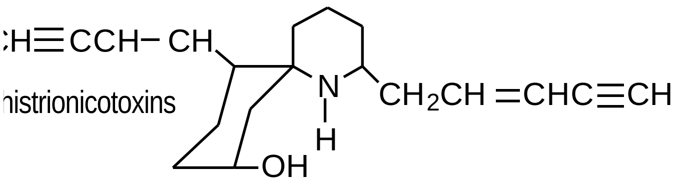
secretion of fluid and electrolytes from the intestinal cells. The result is diarrhea with subsequent dehydration and severe electrolytic loss. Cholera may be self-limiting if not severe, lasting 3 to 6 days. However, if a severe case is left untreated, death may occur in 50% of such cases.

Cholera is spread by contaminated water and foods, especially seafood. On July 29, 1991 the **L.A. Times** reported that 15 cases of cholera had occurred in the United States in the preceeding few weeks. All of the victims seemed to have contracted the disease in Latin America and South America which had been experiencing a serious epidemic during 1991. Most of the 250,000 cholera cases on that continent were localized to Peru. This was the first outbreak of cholera in Latin America since the beginning of the 20th century.

Pertussis or whooping cough is an acute, transmittable disease caused by a toxin produced by the gram-negative bacterium ***bordetella pertussis***. The protein toxin derivatizes one of the subunits of the G-protein in a manner similar to the cholera toxin. Since this material is airborne the effects are seen in the lungs which secrete excessive amounts of fluid resulting in a characteristic cough and the symptoms of the common cold as well as vomiting from the ingestion of mucous.

Both of these conditions are preventable by using hygienic methods and both can be treated prophylactically by immunization. Since infants and small children are most susceptible to pertussis, this immunization is part of the routine schedule of immunizations in the United States.

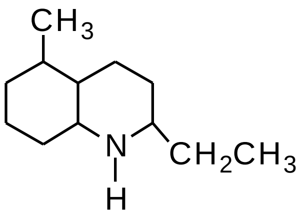
ASIDE: Alkaloid toxins found in dendrobatid frogs



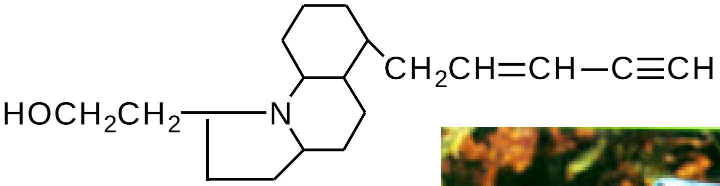
histrionicotoxins

Blocks outflow of K^+ through K^+ channels. This promotes muscle cell contraction and prolongs the release of neurotransmitters.

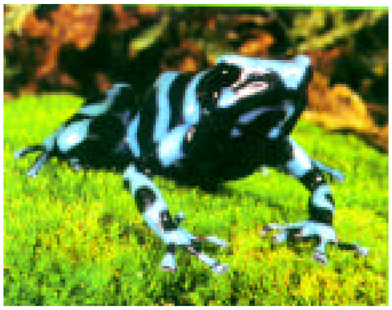
Prevents K^+-Na^+ exchange in channels associated with acetylcholine receptors at neuromuscular junctions



pumiliotoxin C

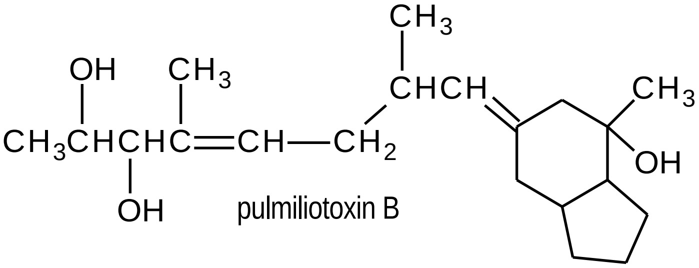


gephyrotoxin



Both of these materials are ion channel/acetylcholine receptor blockers. Their action prevents acetylcholine from stimulating

muscle contraction.



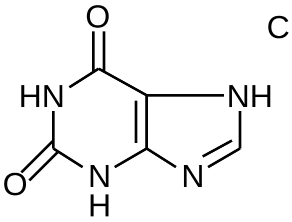
pulmiliotoxin B

Causes release of Ca^{2+} from muscle storage sites; this leads to muscle contraction. Inhibits return of Ca^{2+} to storage sites, prolonging muscle contraction.

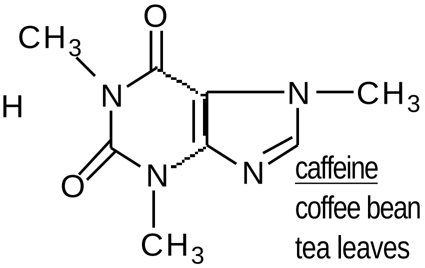
There is some controversy as to whether the frogs produce the toxins themselves as secondary metabolites or whether they are simply processing their food – ants. Dendrobatid frogs raised in captivity lose their toxicity. In fact, chemical analyses of ants similar (but not identical) to those found in the natural habitat show compounds structurally related to the toxins.

(***Scientific American*** February 1983)

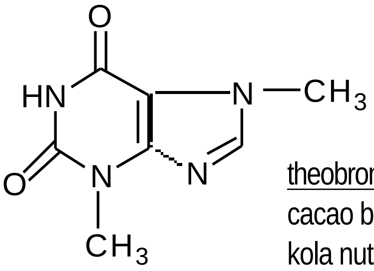
ASIDE: Methyl Xanthines



xanthine



caffeine
coffee bean
tea leaves
cocoa seed
kola nut



theobromine
cacao bean
kola nuts
tea

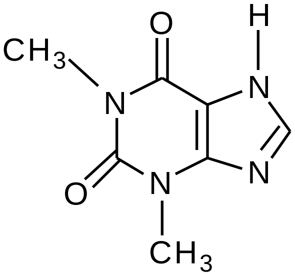
Theoretical explanations for the stimulatory activity of the methyl xanthines.
These compounds inhibit phosphodiesterase reaulting in the prolonged activ of metabolic enzymes.Adenylate cyclase activity is modulated by the bindin free adenosine. The methyl xanthines are antagonists to adenosine but thei bindings do not produce its modulating (inhibitory, attenuating) response. Therefore the end result is stimulatory in relation to cAMP activity.

Effects: relaxation of bronchi, increase in respiration, stimulation of cardiac muscle, diuresis

Methyl xanthines are used in the treatment of asthma.

theophylline or aminophylline (theophylline plus ethylene diamine)

Slo-bid®, Bronkodyl®, Theo-Dur®, etc. available in capsules, caplets, liquids, elixirs, syrups, suspensions.



V. THE ACTIONS AND EFFECTS OF DRUGS AND POISONS

Up to this point we have been discussing pharmacokinetics, that is, the way in which the body acts upon a drug. It is now time to begin a consideration of pharmacodynamics or how a drug acts upon the body. It should always be kept in mind that drugs do not "cure" diseases or conditions. They alleviate symptoms until the body can recover by compensating for a deficiency or excess. Sometimes the body cannot recover and medication must be given for the course of a lifetime. Poisons cause temporary or permanent damage to the body which can result in partial incompacitation or fatality. When considering how drugs and poisons "do their thing" one must be careful to discriminate between the action of the material in question and its effect.

A. Terms

1. **Drug Action** - The action of a drug or poison refers to its biochemical mechanism. How does it interact on a molecular level? A drug may interferewith membrane permeability, for example. Or it may be an enzyme inhibitor, hormone analogue and competitor, or DNA intercalator. Let us consider some specific drug actions.

Penicillin is an antibiotic which destroys bacteria by covalently bonding a transpeptidase enzyme which closes up the cell wall during its biosynthesis. This is its **biochemical mechanism of action**. See Figure 15.

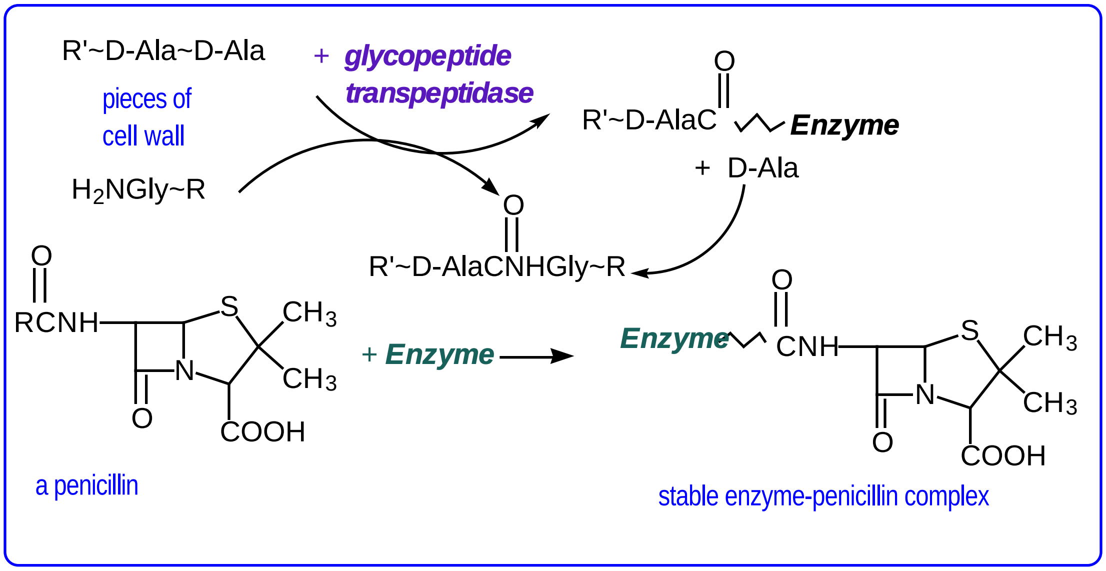
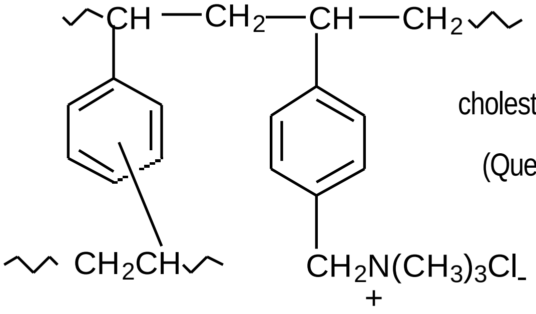


Figure 15
The Action of Penicillins

Acetylsalicyclic acid, aspirin, inhibits the cyclooxygenase-catalyzed first step in the biosynthesis of prostaglandins, prostacyclins and thromboxanes. These latter substances are responsible for the inflammatory and pyretic effects of infection. It is believed that the chemical inhibition reaction involves the acetylation of the enzyme by the aspirin.

A mode of action need not involve enzyme inhibition. It could be a simple **physical action**. Cholestyramine resins are used to form nonpolar aggregates with lipophilic substances. With this action they constitute a good antidote for pesticide poisoning and can serve as a prophylactic in tying up dietary lipids in the intestine so that they will not contribute to atherosclerosis. The pesticides are stored in fat tissue. As the molecules of pesticide are sequestered into the cholestyramine resin more molecules will move from the fat depots in order to reestablish disturbed equilibria in the blood and other body fluids. The resin itself is not absorbed by the intestine but is excreted in the feces.



cholestyramine

(Questran®)

2. Drug Effects

The effects of a drug or poison refer to the observable biological responses such as bacteria dying when the host is treated with penicillin, fever and inflammation subsiding after aspirin therapy or the reversal of toxicity due to pesticide poisoning after a cholestyramine antidote is given. A primary effect is what is sought by the drug discoverer (or poisoner). Secondary effects are side effects, not usually desirable. Sometimes the side effects can be serendipitous such as those seen with sulfinpyrazone in the previous section on gout. Aspirin, used for its analgesic effect, lengthens the time it takes for blood to clot. General anesthetics can cause vomiting.

Sometimes secondary effects can be used therapeutically. Antihistamines, for example, have as their primary effect the alleviation of an immune response. Secondarily, they cause drowsiness and reduce nausea associated with motion. Over-the-counter sleep aides and antinausea preparations contain antihistamines such as dimenhydrinate (Dramamine®), meclizine (Antivert®) and doxylamine (Unisom®).

B. Sites of Action

The sites of action for drugs and poisons generally are either extracellular, on or within a membrane, or intracellular. Extracellular refers to the GI tract before absorption and the blood stream. Extracellular drug

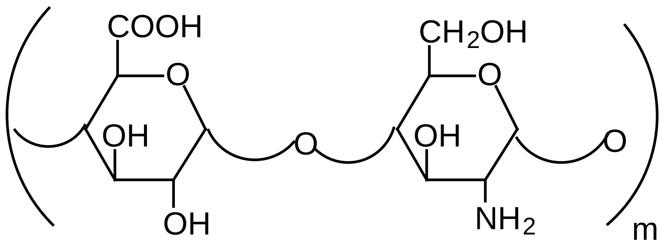
activity may be purely physical such as the cholestyramine sequestration of lipophilic materials mentioned above. The action of laxatives can also be physical. Laxatives loosen the stool by absorbing large quantities of intestinal water and stimulating peristaltic action. Cellothyl® and Colgel® contain methylcellulose a good water absorbant. Metamucil® is psyllium from the Plantago seed. When mixed with an equal volume of water it forms a mucillaginous mass. Caphulac® and Normase® contain lactulose which is poorly absorbed in the intestines and becomes extensively hydrated there.

Extracellular events may also be chemical in nature. Recall that antacids work by the reaction of bicarbonate with stomach acid. See the ASIDE on antacids, laxatives and antidiarrheals.

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2\uparrow$$

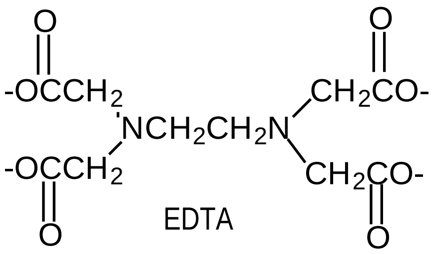
stomach acid — Alka Seltzer — **belch**

The anticoagulant heparin is a polysaccharide sulfate which can form an electrostatic complex with blood-clotting factors (see ASIDE on blood clotting) and prevent the cascade from progressing.



glucuronic acid glucosamine

Heparin
molecular weight 6 - 20 x 10^3
Highly derivatized with sulfate at positions 2 in -uronic acid and 2/3 in -amine



ethylene diamine tetraacetate

Chelating agents such as EDTA tie up heavy metal ions with electrostatic interactions and are used as antidotes in heavy metal poisoning (and I though all I had to do was turn off the radio!).

The membrane of the cell can be disrupted by a variety of nonpolar substances. It is currently believed that this may be the site and mechanism of action of anesthesia gases such as diethyl ether and halothane. If the orderly arrangement of protein receptors and channels is disturbed nerve signal transmission will also be disrupted

and unconsciousness can occur. Many fungicides are bacterial membrane disruptors. And recall that antibiotics like penicillin prevent the cell wall from being completed.

The possibilities for intracellular sites of action are numerous. We have already discussed enzyme inhibition and induction. There are compounds used in cancer chemotherapy which will become involved in DNA and RNA superstructures preventing transcription and translation or replication. Also recall the action of colchichine, the drug used for gout. It interfered with microtubule and microfilament formation necessary for cell proliferation during the inflammatory response of the immune system.

ASIDE 3 - ANTACIDS

Antacids, antidiarrheals, and laxatives are considered topical drugs since they work in the gastrointestinal tract which is considered an extracellular body fluid. These drugs, when used correctly, are not appreciably absorbed through the intestinal wall and do not show systemic effects.

The principal commercial antacids are

Chemical Formulas	Some Brand Names®
$Al(OH)_3$ (antidiarrheal)	***Camalox, Di-Gel, Maalox, Mylanta, Mucotin, Pepsogel, Rolaids***
$CaCO_3$	***Alka-2, Camalox, Chooz, Pepto-Bismol, Titracid, Titralac, Tums***
$MgCO_3$ (laxative)	***Bisodol, Di-Gel, Gaviscon, Marblen***
$Mg(OH)_2$ (laxative)	***Camalox, Di-Gel, Maalox, Milk of Magnesia, Mucotin, Mylanta, Vanquish***
$NaHCO_3$	***Alka-Seltzer, Bisodol, Biroschi, Bromo Seltzer, Eno, Soda Mint***
Na_2CO_3	***Rolaids***

The chemical action of these substances includes neutralization of gastric HCl, which in turn affects the action of pepsin in the stomach and the binding of intestinal phosphate. Laxatives also stimulate the muscles of the lower bowel as well as absorb water themselves. All of the drugs listed above decrease the GI absorption of other drugs and therefore should not be taken with other drugs.

C. RECEPTOR THEORY

In preparation for the next part of this course on the nervous system, we have to consider a very important site of action in and on the cell membrane. It is believed that most drugs act at a specific site called a receptor located within a target cell or in/on its membranes. Receptors are proteins, glycoproteins, lipoproteins, or nucleoproteins in their biochemical makeup. These receptors serve a natural purpose in that they form complexes with natural chemicals and these complexes trigger responses within a cell. A receptor, then, is a chemical entity which interacts with an

endogenous species, or a drug (or poison), resulting in a biological response.

Those chemicals which can cause a **positive response** when combined with a receptor are called **agonists**. An **agonist** is said to have **affinity** (binding) for that receptor as well as **efficacy** (causes a cellular response).

If a species binds to a receptor with **no biological response** ensuing, it is called an **antagonist**. An **antagonist** has **affinity** but **no efficacy**. **Antagonists can compete with agonists for binding to a specific receptor.**

Agonist + Receptor ------> **Agonist-Receptor complex** ⟹ *response*

Antagonist + Receptor ------> **Antagonist-Receptor complex** ⟹ ***no response*** (X)

Receptor theory is the key to understanding why most drugs and poisons produce extensive effects and specific effects even when they are used in realtively minusucle amounts. It is interesting to note that physiological studies of endogenous agonists often followed investigations of exogenous agonists and antagonists. That is, drugs and poisons have been used for millenia before receptor theory was proposed and natural agents were studied. In fact the mechanisms for most of the therapeutic drugs used in the treatment of CNS (Central Nervous System) disorders are unknown .

It was this type of roundabout thinking which led to the discovery of some natural (endogenous) receptor systems which went unexplained for a long time. The best example is that of the opiate narcotic analgesics, that is, morphine and its derivatives heroin and codeine. These drugs and their euphoric, sedative, analgesic, antitussive, constipating effects have been known for since the beginnings of recorded history. Since they are effective in very small doses, it was postulated in the early 1970s that there must be opiate receptors for endogenous opiate molecules. This theory was tested first using a compound which was an opiate antagonist, naloxone. Naloxone will block the effects of opiate drugs and is used to treat overdoses. It was found that during trauma the use of naloxone would increase pain and prevent naturally induced analgesia. It was only a matter of time until the endogenous opiates, the endorphins (for endogenous morphine) and enkephalins, were discovered. Now there exists a whole nomenclature of opiate receptor types and their various effects.

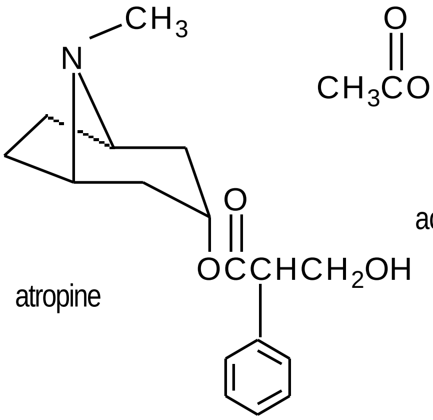
A chronicle of this monumental discovery with all of its characters and intrigues can be found in Anatomy of a Scientific Discovery: The Race to Discover the Secret of Human Pain and Pleasure by Jeff Goldberg, Bantam Books, 1988.

When an agonist or antagonist binds to a receptor it may do so with **specificity** or **selectivity** or both. **Specificity** implies **having only one** site of binding or **biochemical action.** **Selectivity** means that **only one particular effect is observed**.

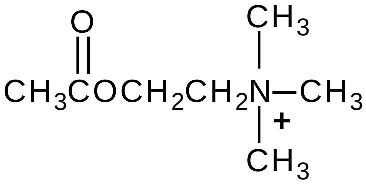
An ideal situation would be to have a drug both with specificity and selectivity. But is this possible? Can we design such a "magic bullet"?

1. Specificity

Atropine is an antagonist for the naturally occurring neurotransmitter (nerve signal carrier) acetylcholine. Its structural similarity to acetylcholine makes it very specific for acetylcholine receptors. How about its effects? - dry mouth, a decrease in stomach acid production, dilation of the pupils, an increase in blood pressure. They are systemic, that is, they lack selectivity because acetylcholine receptors are located in many sites all over the body.

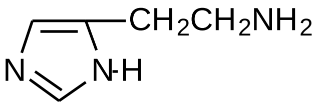


atropine

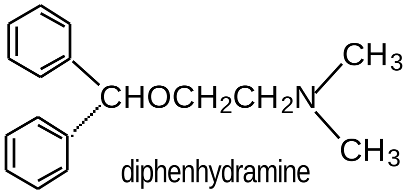


acetylcholine

2. Selectivity



histamine



diphenhydramine

Diphenhydramine is a histamine antagonist (antihistamine) as well as well as an acetylcholine antagonist and is a local anesthetic (affects nerve ion channels). So it is fairly nonspecific. However, in small doses it will exhibit limited effects as an antiemetic and sedative. It has selectivity.

Heparin, the anticoagulant mentioned previously, is one the few drugs with selectivity and specificity.

The method of drug administration or exposure to a poison may impart selectivity to a xenbiotic. For example, atropine can be applied directly to the eye for the dilation of the pupils. Note that eventual absorption into the blood stream from this site will cause systemic effects. Another example would be the topical (skin) application of a local anesthetic such as benzocaine. Monoclonal antibodies, which are specific for chemical functional groups, are tissue and cell-specific natural agents to which drugs can be chemically bonded.

D. The Measurement of Drug Effects

1. Terms

Since it is the effects of a drug or poison which ultimately interest us as human beings, let us now consider how pharmacologists and toxicologists measure and assess the usefulness of an entity. The term dose has been used several times up to now. We know what the term means but let us define it for the record. The **dose** is the amount of a drug needed at a given time at a specific site to produce a desired biological response. The key parts of this definition are the time factor, site (namely the receptor site) and the response which can be therapeutic or toxic. The latter can range from minor undesirable effects to lethal ones, that is, death. For example, aspirin given in low doses is used to combat fever, pain and inflammation due to minor injuries. However, in order to alleviate the massive inflammation process in rheumatoid arthritis, much higher doses must be used to the point of toxic side effects occurring, namely tintinitus (ringing in the ears).

During the course of drug therapy it is important that enough of a drug be given to treat the condition in question and to maintain a maximum concentration at the site of action for continued effective treatment. Figure 16 illustrates the variation in drug concentration in the blood plasma as a function of time and what is happening during its lifetime in the body. The Figure also presents some terms used in pharmacology and to a certain extent in toxicology.

The **minimum effective level** is that concentration of the drug in the blood below which no therapeutic effects are observed. The **therapeutic range** is the blood drug concentration range in which effects will be observed. The **time of onset** is the time it takes to reach the lowest plasma concentration at which effects will be observed. The **peak plasma level** is the time at which the highest concentration of drug will be found in the blood after the administration of a single dose. **Latency** refers to the amount of time from the administration of the drug until it reaches the peak plasma level. And finally the **duration** of drug activity is the length of time that desired effects are observed.

In addition to the terms in Figure 16 we also will be concerned with the **potency** of drugs and poisons. What do you think of when someone tells you that a spice you ate was potent? Potency is a comparative term and refers to the dose required to produce a particular effect relative to a standard. If we were comparing local anesthetic effects for three different drugs we would first have to set a standard degree of numbness - say, 50% relief (either 50% anesthesia in one subject or complete anesthesia in 50% of a stated population). Then we would vary the doses of the three drugs until we achieved the same degree of anesthesia (50%) for all three. Let us use cocaine, lidocaine and procaine. For cocaine the dose leading to the standard effect would be 2 mg/mL, for lidocaine 4 mg/mL and for procaine

10 mg/mL. Which the most potent? The cocaine is, of course. We used the least amount to get our desired effect.

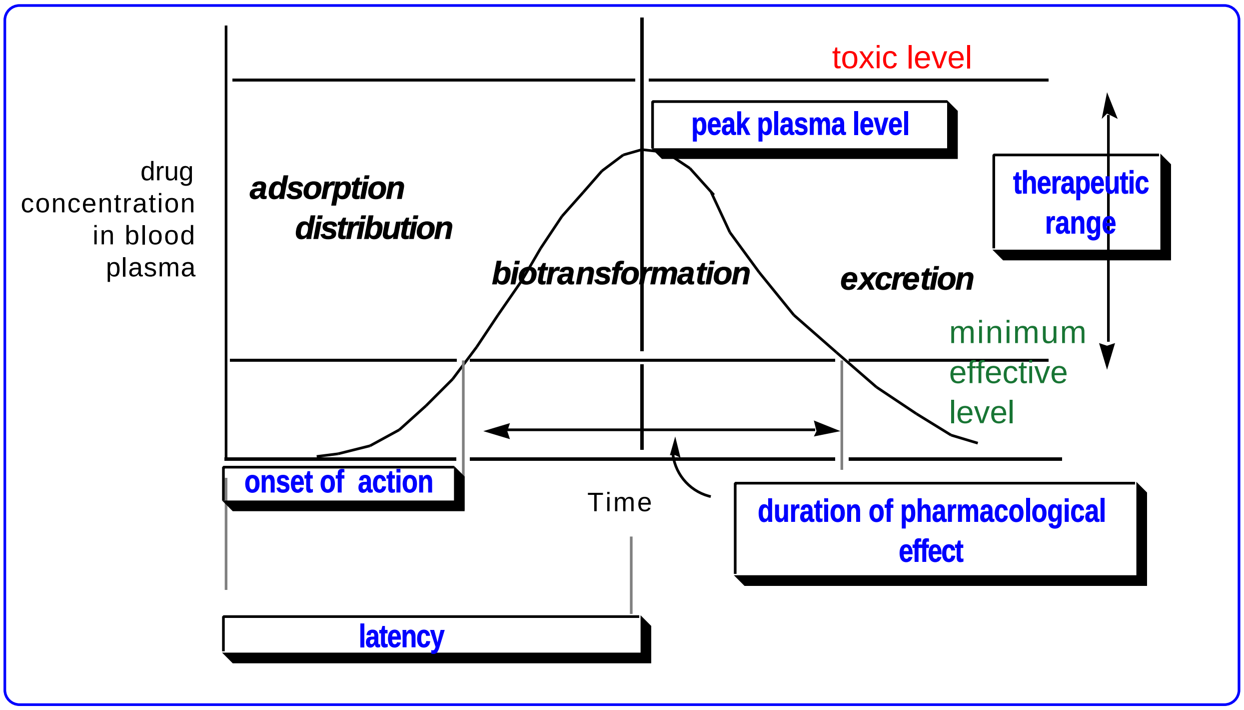


Figure 16
Variation of Drug Concentration in the Blood with Time
Key Terms

In the previous paragraph we saw that in order to make some comparisons of drugs we have to define a population of patients or victims (in the case of poisoning). When investigating a new drug certain fundamental tests are designed based upon specific populations and statistical data is generated concerning the therapeutic and toxic effects. Then these data are combined into various indices which will lead to someone, somewhere weighing the relative risk-to-benefit ratios and deciding whether the drug should be marketed or what EPA standard should be set for acceptable safety in the use of commercial and agricultural chemicals. This last stage is subject to the greatest controversy as you might conjecture. It is called Risk Assessment and it infiltrates not only pharmaceutical decisions but also political decisions when applied to toxic waste dumps, air and water pollution, and the use of agricultural pesticides.

2. The Concept of Equivalence

When is a generic drug equivalent to a proprietary one? Can a drug be substituted for another if it falls within the same therapeutic, pharmacological or structural class? This are legitimate questions which must be answered frequently by doctors, pharmacists and other health professionals. According to the Merck Manual (15th Edition) there are three

types of equivalence to be considered: **chemical, biological, and therapeutic**. Let us look at each.

Chemical or pharmacological equivalence - Two or more drug products which contain the same chemical compound in the same amount in two or more dosage forms and meet official FDA standards are said to be chemically equivalent. Note, however, that the "inactive" ingredients may be different.

Bioequivalence - Two chemically equivalent drugs which, when administered to the same patient in the same dose regime, produce an equivalent blood/tissue concentration are bioequivalent.

Therapeutic equivalence - If two or more drugs produce the same therapeutic effect or the same toxicity they are therapeutically equivalent. Note that they might not be bioequivalent.

If the therapeutic range of a drug is broad then many substitutions can probably be made. This occurs, for example, with the penicillins. However, if there is a narrow therapeutic range then bioavailability must be carefully considered. Examples of this situation are the use of digoxin (cardiac stimulant) and phenytoin (anticonvulsive).

When determining bioequivalence the curves seen in Figure 16 are compared for each drug. If there is an exact overlay, you are looking at bioequivalence. Should the shapes of the curves differ but the magnitudes remain the same, then the extent of availability is equivalent but one of the factors going into the curve - absorption, biotransformation, distribution, or elimination - must be investigated.

The dosing level of a drug should be about equal to its elimination half-life. This allows a more consistent level of drug in the blood over time.

Figure 17 presents some hypothetical information graphed as the dose of a drug versus the number of people who respond in a standard way. This is called a dose-response curve. Notice that the x-axis can be either dose or the log dose (powers of ten). The units of dose will depend upon the type of material you are studying - drug, poison, air pollutant, water pollutant. Certain values are chosen from the curves and compared. Please note that this relationship can be graphed for any type of drug or poison you might want to investigate. The following are some common points of reference and the ratios which are used.

Median refers to the point at which 50% of the stated population responded.

ED_{50} the median effective (therapeutic) dose - units of mg/kg body weight

LD_{50} the median lethal (fatal) dose - units of g/kg, mg/kg, mg/kg

LC_{50} the median lethal concentration - units of ppm, mg/L

TD_{50} the median toxic dose

CD_{50} the median convulsive dose

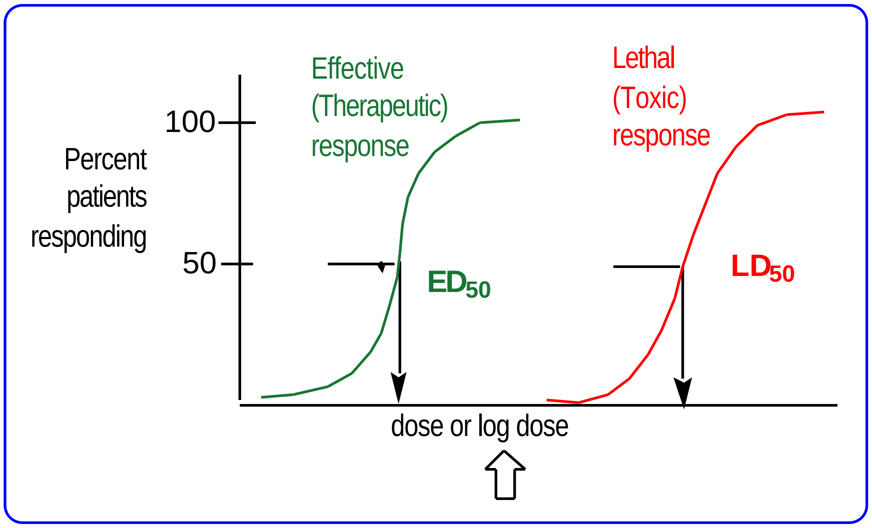


Figure 17
Dose-Response
Curve

Note that you can pick any response that you would like to study - analgesia, anesthesia, nausea, rash, etc. It is also important that the route of administration or exposure be included with the numerical information as well as the animal used to testing. So a typical entry in a reference like the Merck Index might read - for Labetalol - LD_{50} in rats, mice (mg/kg): 4000, 1450 orally; 107, 114 i.p.; 53, 47 i.v. Be sure that you understand what each entry means.

The combination of this type of data leads to terms for risk assessment such as

$$\text{Therapeutic Index (TI)} = \frac{LD_{50}}{ED_{50}} \qquad \text{Certain Safety Factor (CSF)} = \frac{LD_1}{ED_{99}}$$

$$\text{Standard Safety Margin (SSM)} = \frac{LD_1 - ED_{99}}{ED_{99}} \times 100$$

In determining an optimal dose for a drug the idea is to pick one for which the toxic effects would occur in a minimum number of people and the therapeutic effects would occur in the maximum number. With individual variations there is no way that the same effects will be seen to the same extent in two random samples. The most realistic view is that the drug dose will keep a patient within a range of therapeutic/toxic effects. See Figure 18.

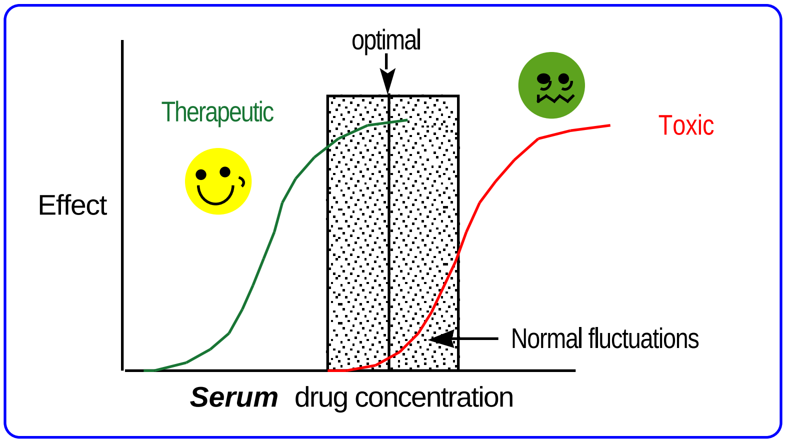


Figure 18
Optimal Dose
Reference: N. Eng. J. Med 307 286-290 (1982)

E. TOXICITY

Toxicity can be complex and deserves some special consideration. As previously mentioned a toxic response can be anything from nausea or a rash to death. All too often the public translates the term as the last - a lethal effect. There are degrees of toxicity, the two major categories being acute and chronic.

Acute toxicity refers to a chemical's ability to do systemic damage in a large-dose one-time exposure. Chronic toxicity is a chemical's ability to do systemic damage over a relatively long period of time, in a series of low dose exposures. These are definite distinctions since acute and chronic toxicity may not elicit the same symptoms or affect the same organs or areas of the body. For example, acute toxicity from chloroform ($CHCl_3$) or carbon tetrachloride (CCl_4) has central nervous system effects - excitability, dizziness, narcosis. Chronic toxicity results in long term liver damage.

Another example is lead poisoning wherein acute toxicity symptoms are seen in the GI tract. Chronic toxicity causes damage to blood cell formation and musculature and subtle decreases in mental ability. (See ASIDE #1) Frequent exposure to large doses can show both types of effects - this is called subacute.

The route of exposure will also vary the toxicity. The skin is relatively impermeaable to many substances while the placental membrane is almost completely porous.

Human toxicity data, especially the median lethal dose, is extrapolated from animals or from accidental poisoning, homicides and suicides. Extrapolations from animal data are educated estimates which consider the differences in species and building in a safety factor. If a lethal dose is 10 mg/kg in a rat and we consider a human to be 10 times more sensitive 1 mg/kg will have another 10-fold safety margin. Animal testing also involves using what may seem as ridiculous doses in order to cover the safety factor. To find a statistically valid effect which occurs once in one million subjects, several million animals would have to be used, which is exhorbitantly

expensive. So the experimentation might involve increasing the dose one million times to a lesser number of animals. Is this valid?

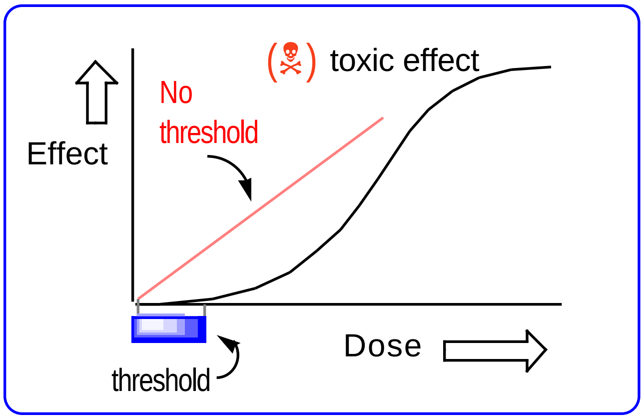


Figure 19
Threshold Concept

Important to the determination of risk is the threshold dose of a toxicant. Threshold is the amount of a material below which you will see no toxic effect. See Figure 19. For most drugs and some pollutants there are lower limits to the degree of toxicity. In fact, it is known that small amounts of metals, for example, are essential to proper growth and development while larger quantities are dangerous. Too much water will drown you. But what about toxic effects such as those seen in cancer? Is there any amount of a carcinogen which can be administered without it causing a toxic effect? There are two schools of thought on this very important scientific, moral and political issue. One is that there is a threshold and the other is that there is no threshold, that is, any amount of a carcinogen can cause cancer (cancer is a unimolecular event). These arguments become very pertinent when we consider the extremely low levels of detection to which modern instrumentation is taking us - nano-, pico-, atto- gram levels. Confusing the issue is the presence of natural materials which can cause cancer. Even if we eliminate every shred of technology, there will still be toxic and carcinogenic substances which nature has devised. How much is too much? How little is too little? The debate continues.

F. CHEMICAL SENSITIVITY

A topic which has merited publicity in recent years is chemical sensitivity or the immune response to environmental chemicals. This sensitivity can be present for something like the sulfites used in preserving vegetable especially lettuce to allergic reactions to anything and everything synthetic. It is a relatively new field and is subject to the expected array of investigators and disparagement.

G. DRUG INTERACTIONS

When polypharmacy is necessary, the drugs which are prescribed may have effects on the body which may be greater or less than any one taken by itself. In addition, the drugs may have interactions with the constituents of the diet. The following are the general types of interactions which may occur and a brief discussion of what action is causing them.

1. Interactions which produce greater effects

Additive - the drugs have the same biochemical mechanism and will react with the target cells as long as receptor sites are available. Examples are the cyclooxygenase inhibitors (prostaglandin synthesis inhibitors) aspirin and acetaminophen (Tylenol®), antihypertensives propanolol and the rauwolfia alkaloids.

Summation - different mechanisms of action are involved with the same effects for each. Analgesia can be achieved by stimulating opiate receptors (codeine) and blocking prostaglandin synthesis (aspirin).

Synergism - a potentiation or prolongation which results in much greater than expected effects. This could involve competitive substrates for an enzyme or receptor, decreased excretion, displaced plasma protein binding, etc. The analgesic propoxyphene (Darvon®) slows down the excretion of ethanol and so increases the depressant effects of the alcohol. Recall the example given earlier of the monoamine oxidase inhibitors used as antidepressants and the tyramine-containing foods which could precipitate a hypertensive crisis.

2. Interactions which produce diminished effects

All of the above represented greater than usual effects. Drugs can also have diminished effects due to various interferences. These lesser effects may be physiological, pharmacological, biochemical or chemical.

Physiological - happening at different sites and counteracting each other. Diazoxide, used for hypertension, blocks insulin release from the pancreas and so has a hyperglycemic effect. Insulin, on the other hand, lowers blood glucose and is hypoglycemic.

Pharmacological - actions at the same site. This is the typical agonist-antagonist competition for the same receptor. Atropine blocks the binding of acetylcholine. Naloxone blocks the binding of morphine.

Biochemical - somehow the drug has decreased availability. This is the effect of induction of the liver microsomal enzymes. The metabolism of

certain drugs is accelerated and less is available for therapeutic use. There may also be an increased excretion rate or competition for transport across membranes which put less agonist at its respective receptor site.

Chemical - complex formation. Earlier we discussed the complexation which can occur between the chelating agent EDTA and heavy metals or the sequestration of lipids in cholestyramine resins. The immune system has antibody-antigen complexes formed which can help in the elimination of the antigen (xenobiotic).

Drug resistance, especially to cancer chemotherapeutic agents, is an interesting phenomenon which is gradually yielding to intense research. It may be due to a breakdown in biotransforming pathways or the cell could be pumping the drug out as fast as it comes in.

Within the last few years there have been some intesting example of drug interactions not only with other drugs but with foods as well. For example, in late 1990 a warning was issued to those using nicotine gum in anti-smoking programs. Consuming coffee or carbonated beverages prevented the absorption of nicotine in the mouth. (Swallowing nicotine causes GI upset and hiccups.) In early 1991 it was found that consuming grapefrut juice with the experimental antihypertensive drug felodipine, resulted in a three-fold increase in the drug concentration in the blood. This caused several unpleasant side effects including increased heart rate and dizziness.

Familiarity with the terms presented in this section can help to clarify much information and misinformation or misinterpretation which can occur in the media concerning drugs and environmental issues. Each one of these topics could be studied in more depth but it is hoped that the groundwork has been laid for the rest of the course.

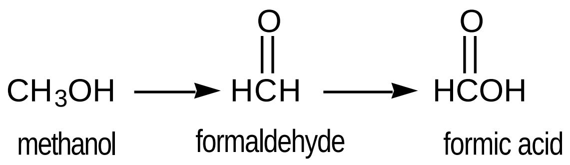
VI. POISONING AND THE USE OF SPECIFIC SYSTEM ANTIDOTES

The percentage of ingestions for which a specific antidote (or "antipoison") does exist is small. Where a specific antidote can be used, it is vital that it be administered as early as possible with close attention to the recommended dosage. Antidotes can be categorized as to their mechanism of action.

One of the primary goals in the immediate treatment of poisoning is to prevent the toxic material from being absorbed and/or to make it less concentrated. First aid can include, therefore, (upon the advice of a physical or poison control center), administering water, charcoal, milk, or Epsom salts, or the ingestion of syrup of ipecac to induce vomiting. The last treatment must be undertaken only with expert medical advice of a physician as many toxicants, such as petroleum products, could be inhaled during vomiting. This would lead to chemical pneumonia.

A. ENZYME INHIBITION AND INDUCTION

Antidotes can interfere with the metabolism of the poison either by blocking the metabolism of the active poison or by accelerating the metabolism of the poison to a nontoxic form.



The administration of ethanol to a patient suffering from methanol intoxication will competitively inhibit the metabolism of methanol to formaldehyde and formic acid.

It should be noted that formic acid and formaldehyde are respectively 6 and 30 times more toxic than methanol.

The administration of sodium thiosulfate (12.5 grams; a 25 percent solution administered intravenously at a flow rate of 2.5-5mL/min over a 10-minute period of time) will result in the conversion of the much more toxic cyanide to its less toxic thiocyanate form. This treatment of cyanide poisoning with sodium thiosulfate should follow the use of sodium nitrite. The administration of both the sodium nitrite and sodium thiosulfate is dependent upon the hemoglobin of the patient. The Fe^{2+} form of hemoglobin will also be oxidized by the sodium thiosulfate and sodium nitrite to the Fe^{3+} form (methemoglobin). This oxidized form binds cyanide readily to form a stable complex which can be metabolized. See ASIDEon CYANIDE.

B. ACCELERATION OF EXCRETION

Antidotes can accelerate the excretion of the poison.

The administration of sodium or ammonium chloride at a dose of 6-12 grams/day orally or the equivalent as normal saline every 6 hours intravenously will enhance the excretion of bromide-containing compounds.

C. ANTAGONISM

Antidotes can block the receptor site of action of the poison.

The administration of atropine at a dose of 1-2 mg (for children under 2 years, 1 mg or 0.05 mg/kg) intramuscularly or intravenously repeated every 10-15 minutes until atropinization is evident will effectively block the receptor site of action of anticholinesterases such as the organophosphate insecticides and neostigmine, physostigmine and pyridostigmine. The administration of atropine is usually followed by the use of pralidoxime

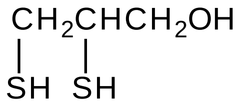
chloride 25-50 mg/kg (1 grams in adults) intravenously. This dosage is repeated in 8-12 hours as needed.

Antidotes can compete with the poison for the receptor site of action.

The administration of 100 percent oxygen via inhalation for no more than two hours followed by the inhalation of room air is the usual accepted antidote for carbon monoxide intoxication. Other examples of this mechanism of antidotal action would be the administration of naloxone (Narcan®) for opiate intoxication, and the usage of vitamin K preparations for oral anticoagulant overdose.

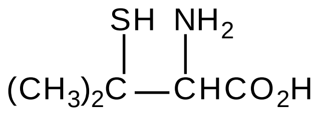
D. CHELATION

Antidotes can chelate the poison, thus forming a nontoxic complex.



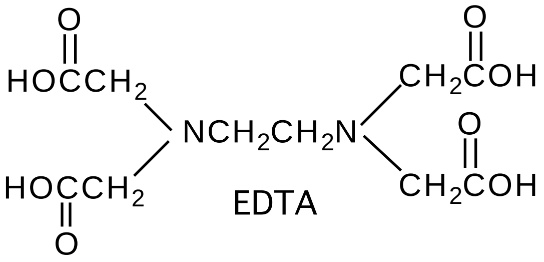
dimercaprol (BAL)

Examples of this mechanism of action include the administration of BAL (Dimercaprol) for the treatment of arsenic, copper, lead, or mercury intoxication. BAL is usually administered in a does of 3-5 mg/kg intramuscularly every 4 hours for 2 days, then every 4-6 hours for an additional 2 days, then every 4-12 hours for up to 7 additional days.



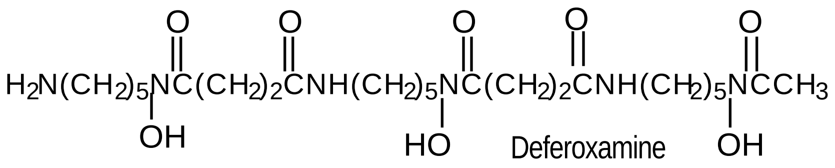
penicillamine

The administration of the chelator pencillamine has been shown to be effective in the management of copper, lead, or mercury intoxication. Penicillamine is administered orally at a dose of 100 mg/kg/day (maximum 1 gram) in divided doses for up to 5 days. If long-term therapy is to be used, the dose of 40 mg/kg/day should not be exceeded.



EDTA

EDTA has been shown to be of value in the therapy of lead intoxication. It can be administered either deep intramuscularly or by slow intravenous infusion. The dose of 75 mg/kg/day should be administered in 3-6 divided doses for up to 5 days; this dosage regimen may be repeated for a second course after a minimum of 2 days.



Deferoxamine

Deferoxamine (Desferal®) has a specific ability to chelate iron and is useful in treating acute iron intoxication. Deferoxamine can be administered

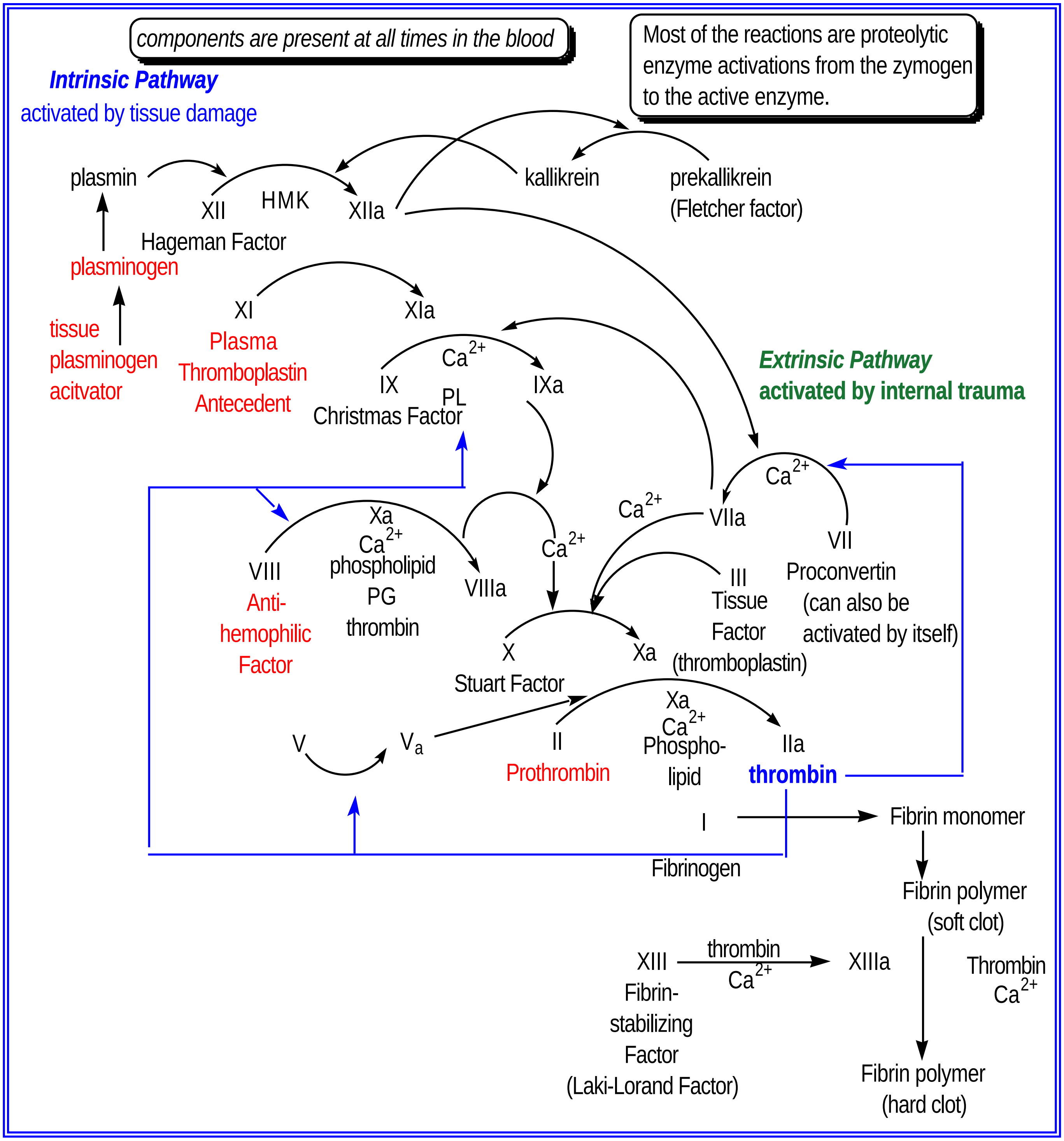
either intravenously or intramuscularly. The severity of the intoxication will determine the route of administration. A dose of 500-1000 mg is given intramuscularly as the initial dose; this is followed by a dose of 250-500 mg every 4 hours for a total of 80 mg/kg in the first 24 hours. On subsequent days a dose of 250-500 mg can be administered every 4-12 hours. If administered orally, 1 gram of deferoxamine will combine with 85 mg of ferric iron.

E. BYPASS OF ACTIVITY

Antidotes can restore function by repair or bypassing the effect of the poison.

In the case of an acetaminophen overdosage, the administration of N-acetylcysteine (Mucomyst®) is a loading dose of 140 mg/kg orally (dissolved in a suitable vehicle such as juice) followed by a dose of 70 mg/kg every 4 hours for 17 additional doses will prove to be lifesaving. By restoring the sulfhydryl content of the liver that was depleted by the reactive intermediates involved in acetaminophen metabolism, acetylcysteine has been proven effective in the prevention of the acetaminophen-induced hepatoxicity. Other examples of antidotes that function by this mechanism include the administration of folinic acid to antagonize the toxicity of methotrexate and the administration of methylene blue to antagonize the effectiveness of methemoglobin-forming compounds.

Aside 4 - BLOOD CLOTTING CASCADE



BLOOD CLOTTING CASCADE (continued)

Blood Coagulation Factors

Reference: Brewer, M. and Scott, T., Concise Encyclopedia of Biochemistry, Walter deGruyter Publishing Co., New York, 1983; Voet and Voet Biochemistry 2nd Edition John Wiley Publishers 1995.

No.	Name	Properties and Function
I	Fibrinogen	MW 330 kD; composed of 6 chains.
II	Prothrombin (IIa is thrombin)	MW 66 kD, 582 amino acids with 12 disulfide bridges. Glycoprotein. Inhibited by antithrombin III, 2-macroglobulin, 1-antitrypsin and hirudin.
III	Tissue factor, thromboplastin	MW 30 kD; A lipoprotein.
IV	Calcium ions	
V	Proaccelerin; Va is accelerin	249kD; Labile modifier protein.
VII	Proconvertin	46 kD; Labile, activated by tissue trauma.
VIII	Antihemophilic factor	265 kD; Labile modifier protein; absence - Hemophilia A.
IX	Christmas Factor	MW 47kD; single-chain glycoprotein; absence - Hemophilia B.
X	Stuart factor	50kD; a glycoprotein composed of a light and a heavy chain.
XI	Plasma thromboplastin antecedent	136kD; a glycoprotein composed of two similar or identical polypeptides joined by a disulfide bond(s). Inhibited by antithrombin III, trypsin inhibitors(α-1-trypsin inhibitor and CI inhibitor.)
XII	Hageman factor	67kD; single-chained glycoprotein. Inhibited by antithrombin III, C1 esterase inhibitor and lima bean trypsin inhibitor. Inhibition by antithrombin III is accelerated by heparin.
XIII	Fibrin-stabilizing factor (Laki-Lorand factor)	α-globulin. MW 301kD; 4-chains
	Prekallikrein	69kD
	High Molecular Weight Kininogen (HMK)	70kD

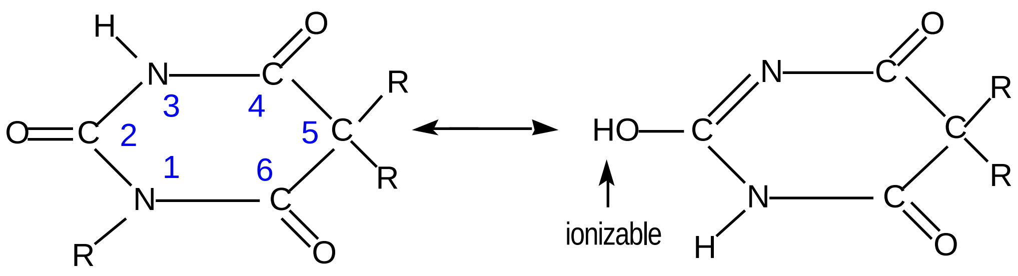
VII. STRUCTURE-ACTIVITY RELATIONSHIPS

How do pharmacological chemists design new drugs? agricultural chemists design new pesticides? testing laboratories study possible carcinogens?

As you might suspect a great number of drugs and poisons can be isolated from natural sources. These were the materials of ancient folk medicine and witch doctors, of healing and war. With the advent of modern chemistry the structural nature of each compound could be analyzed atom by atom and similar structures (analogues) could be constructed and tested for their activity.

The current pharmacological and toxicological age includes the use of computers to investigate molecular environments and predict the activity of a proposed molecule before it is laboriously synthesized by humans or before it can cause irreparable damage to an organism or ecosystem.

A. Barbiturates



1. **Uses** - a. sedative b. hypnotic c. antianxiety

2. **Specific Modifications**

Position	Modification	Result
C-2	S (thiobarbiturates)	Increased lipophilicity
C-5	Large aliphatic groups >7 carbons	Greater activity; shorter duration convulsant activity
C-5	Polar groups	Decreased lipophilicity; abolished hypnotic activity
N-1	Methyl group	Increased lipophilicity; shorter duration

2. General alterations and results

An increase in lipophilicity results in

a. decreased latency
b. accelerated biotransformation
c. increased hypnotic potency

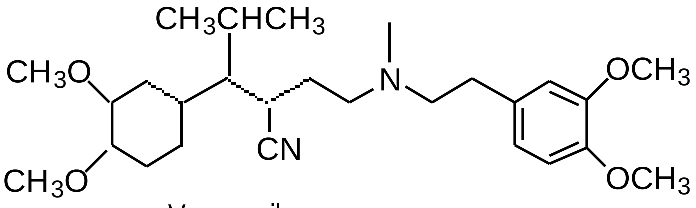
B. Antiischemic, Bradycardic Compounds

1. Uses

Treatment of myocardial malfunction and tissue damage. It is desirable to induce a slower heartbeat (bradycardia) in order to reduce O_2 consumption without causing a reduction in contractile force. Existing drugs (calcium channel blockers) could also reduce the contractile force of myocardial tissue which is undesirable.

2. Goal

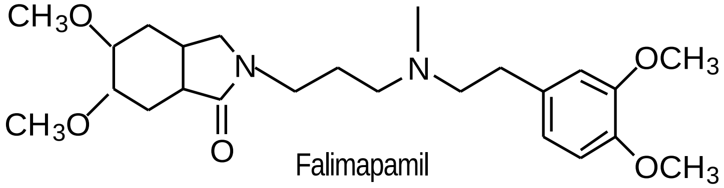
To reduce heart rate without a decrease in aortic blood pressure or contractility and have a longer duration of action than existing drugs.



Verapamil
model compound

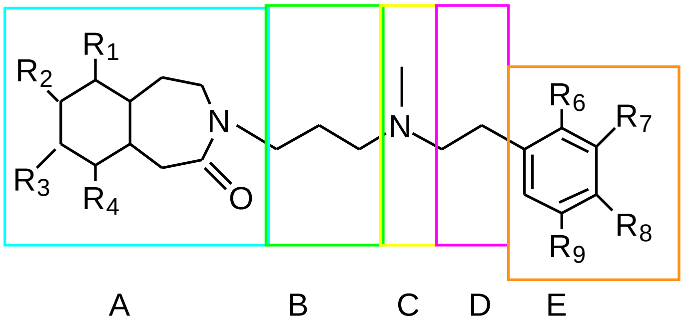
Cyclizing the cyano portion produced a prototypic compound which reduced the heart rate and showed no significant b-blocker activity.

The activity of this compound was different than that of the Ca^{2+}-channel blockers.



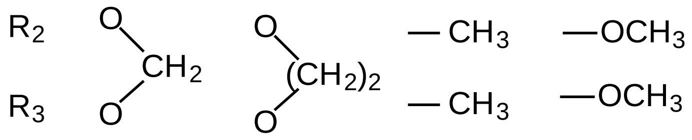
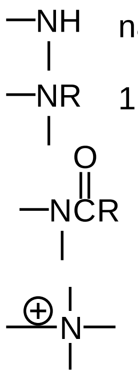
Falimapamil
prototype

They found that increasing the ring size enhanced activity with a 7-membered ring being optimal.



Areas of molecule which were varied.

Administration: 5mg/kg i.v. - assessment of bradycardic activity 5 minutes later.

A		Retention of activity: disubstitution best -OH loss of activity
B	had to have three carbons and be unsubstituted	
C		natural metabolite; slightly more active 1 to 3 carbons, unsubstituted loss of activity; need a basic nitrogen
D	n = 2 to 5 all active with n = 3 optimal N and S substitution were okay; similar activity to n = 3 O more potent R_6 - R_{10} as Cl showed a significant decrease in activity	

The material above is from an article which appeared in the Journal of Medicinal Chemistry 33, 1496-1504 (1990) as presented by Anjali Morey in Chemistry 378 (Biochemical Pharmacology) Spring 1990.

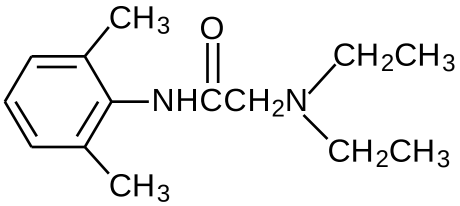
Anjali received her M.D./Ph.D. from UC Irvine in May 1998.

C. Design of a Cardiac Stimulant

1. **Uses**
a. Antiarrhythmic
b. Prevent post myocardial infarction (ectopic beats)

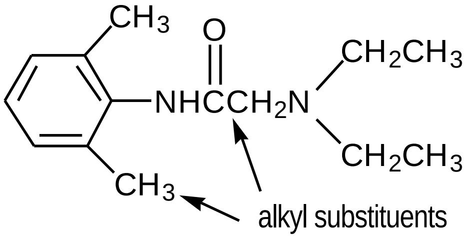
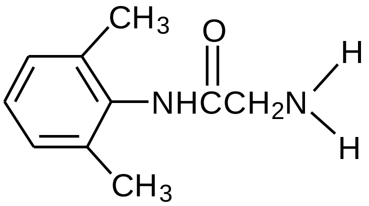
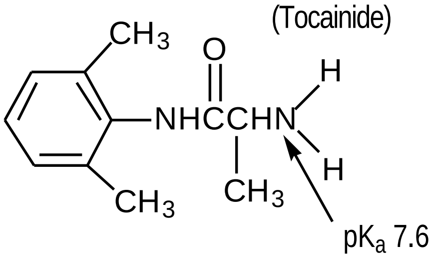
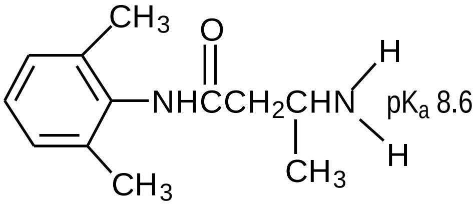
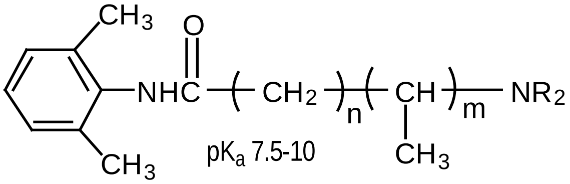
2. **Considerations**
a. Intravenous administration
b. Rapid biotransformation (1/2 life 1.5-2 hours)
c. CNS toxicity

3. **Drug design goals**
a. Oral administration
b. Longer 1/2 life
c. Separation of CNS toxocity and antiarrhythmic acitivty
d. Patentable



model compound is Lidocaine

4. **Potential Drug Design**

Concept	Compound	Pharmacological Effects
steric hindrance to biotransformation		· increased lipophilicity · increased CNS toxicity
alteration in activity of metabolites		· 10% antiarrhythmic activity of lidocaine · longer 1/2 life · lower CNS toxicity
lower rate of biotransformation		· 1/3 antiarrhythmic potency of lidocaine · reasonable 1/2 life · low CNS toxicity · 90% eliminated unchanged
Increase in pKa		· ~90% potency of lidocaine · longer 1/2 life · no CNS toxicity
Variations in pKa and lipophilic nature		***Work of Dr. Eugene Byrnes, Assumption College Worcester, Mass.***

Conclusions: Increase in lipophilicity - increase in potency; Increase in pKa - decrease in toxicity

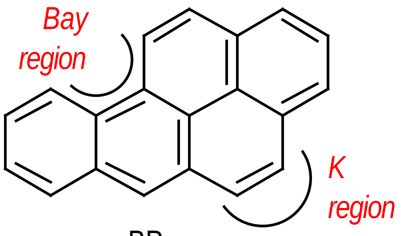
D. Computer-Assisted Drug Design

Polycyclic Hydrocarbons

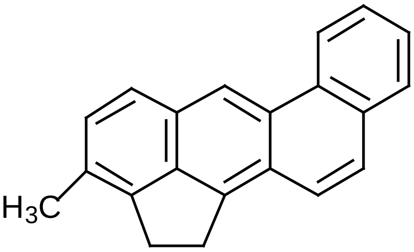
1. **Function** - Carcinogens

2. **General considerations**
a. four to six fused aromatic rings
b. unsubstituted C=C bond bordered by two aromatic rings (K-region)
c. positions of methyl substituents

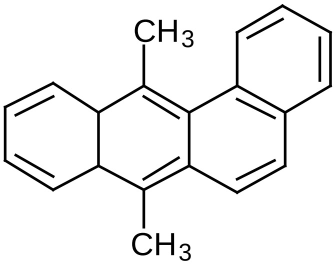
3. **Examples**



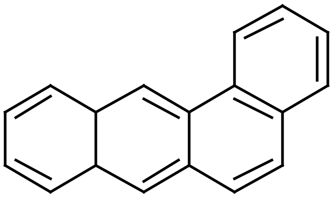
BP
(benzopyrene)

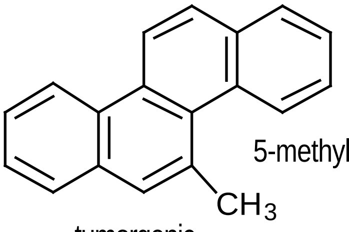


3-MC
(3-methylcholanthrene)

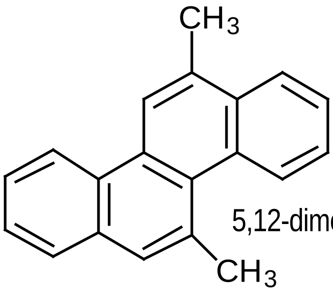


DMBA
one of the most potent carcinogens known


benzanthracene
very weakly active



5-methylchrysene
tumorgenic



5,12-dimethylchrysene
little activity

4. **Predictors of Carcinogenicity Using Molecular Descriptors**

a. Physiochemical - density, melting and boiling points, molecular weight, partition coefficient

b. Topological - number of carbon atoms and rings, substructures and their environments, branching

c. Geometrical - molecular shape, volume and surface area

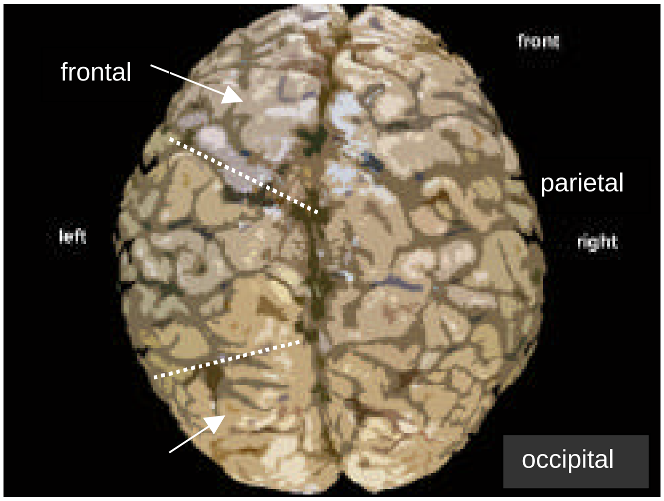
d. Electronic - electron density and affinity, pi bond reactivity, dipole moment, ionization potential

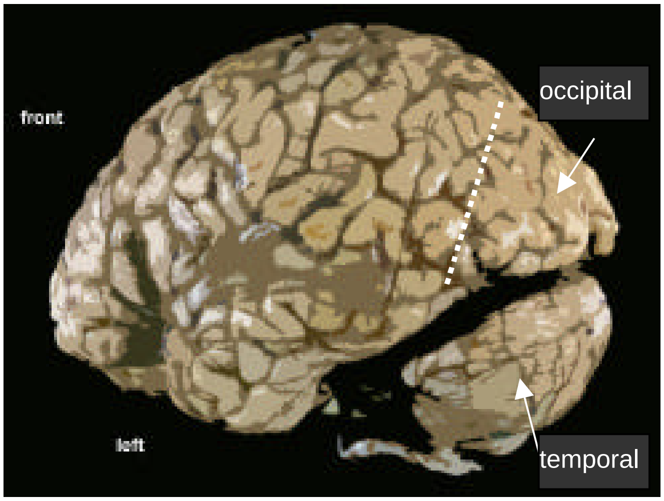
DRUGS AND POISONS AFFECTING THE CENTRAL NERVOUS SYSTEM

Recall once again that the nervous system is an integrated whole. No one part can act entirely independently. The subdivisions which we are discussing, that is, the peripheral and central nervous systems, are anatomical and functional partitions which we use in order to make learning about the nervous system a little more organized. Keep in mind, however, that the neurons of the central nervous system are protected by the bony casing of the skull and spinal column and that chemicals attempting to reach the CNS through the bloodstream must pass the blood-brain barrier.

A. Anatomy Of The Brain

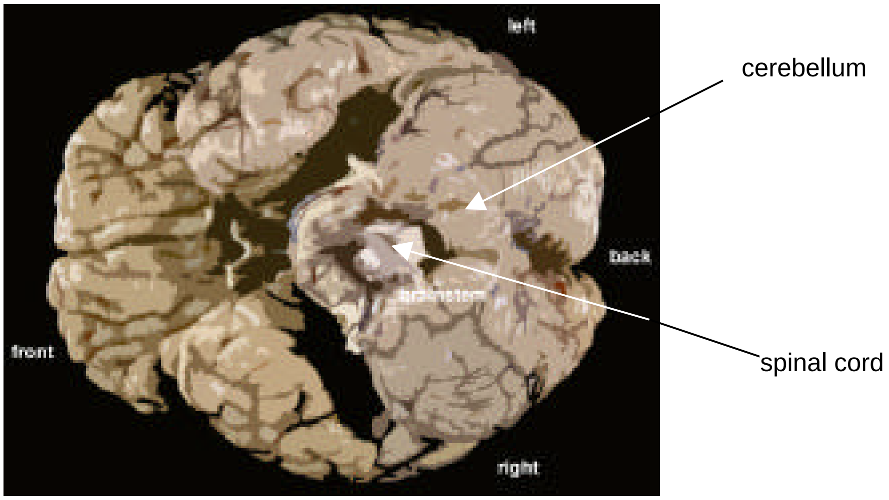
The anatomy of the brain and spinal column is very complex. The outer, most visible portion of the brain is known as the **cerebrum**. The cerebrum accounts for about 80% of the mass of the brain. Covered with a layer of grey matter known as the **cerebral cortex**, the cerebrum is divided into two hemispheres - left and right.





Each hemisphere, in turn, has four lobes: frontal, parietal, occipital, and temporal (working from front to back and then under).

Four ventricles bathe the brain in **cerebral spinal fluid (CSF)** and a host of blood vessels supply nutrients and carry away metabolites. See Figure 27 a. The back and lower portions of the brain are made up of the **cerebellum**, the **pons**, the **midbrain**, and the **medulla**.



Basal ganglia lie beneath the cortex and have effects on muscle tone and posture especially in involuntary motor movement .(This is called the **extrapyrimidal motor system**. The voluntary motor system is referred to as **pyrimidal**.)

The neurons of the frontal lobes direct motor functions including the sequencing of physical movements, they influence emotional behavior and personality including the development of inhibitions and correct social behavior, and they contribute to the processing of expressive speech.

The parietal lobes are responsible for integrating the sensory input from touch, taste, smell, etc. The complex organization of this section of the brain allows us to recognize patterns in our experience, maintain physical orientation and stability, and perform more intricate intellectual tasks such as math and chemistry.

The vision center is located in the occipital lobes. It is there that visual images are coalesced into meaningful wholes.

The temporal lobes seem to be the seat of human individuality. Hearing, memory, vision, a sense of time, and verbal comprehension are coordinated in the temporal lobes. This area also is the seat of emotions such as jealousy, anger, happiness, and fear.

Again, keep in mind that the control attributed to each lobe mentioned above is not exclusive of the other lobes because of the integrated network of neurons connecting various portions of the brain. In addition there are other distinct bodies or diffuse neuronal systems which lie within the lobes just described.

The **thalamus** lies at the approximate center of the brain and offers interpretation of sensory input leading to appropriate responses. It is regulates the state of consciousness (alertness and attention). Beneath the thalamus is the **hypothalamus** which is the principal integrating system for the autonomic nervous system, cardiovasculature, body temperature, fluid volume of the body, the gastrointestinal tract, overall metabolism, and sexual and circadian cycles. Both of these bodies can secrete hormonal substances which aid in the regulation of tissues and organs throughout the body. The **limbic system** surrounds and includes the thalamus and hypothalamus as well as other subsystems such as the amygdaloid complex, hippocampus, and septal area. It is associated with complex emotions such as fear, feeding and mating. Emotion is linked to the **amygdala** while short- and long-term memories are established in the **hippocampus**. (see Figure 27b)

The **cerebellum**, a part of the hindbrain, is in control of voluntary

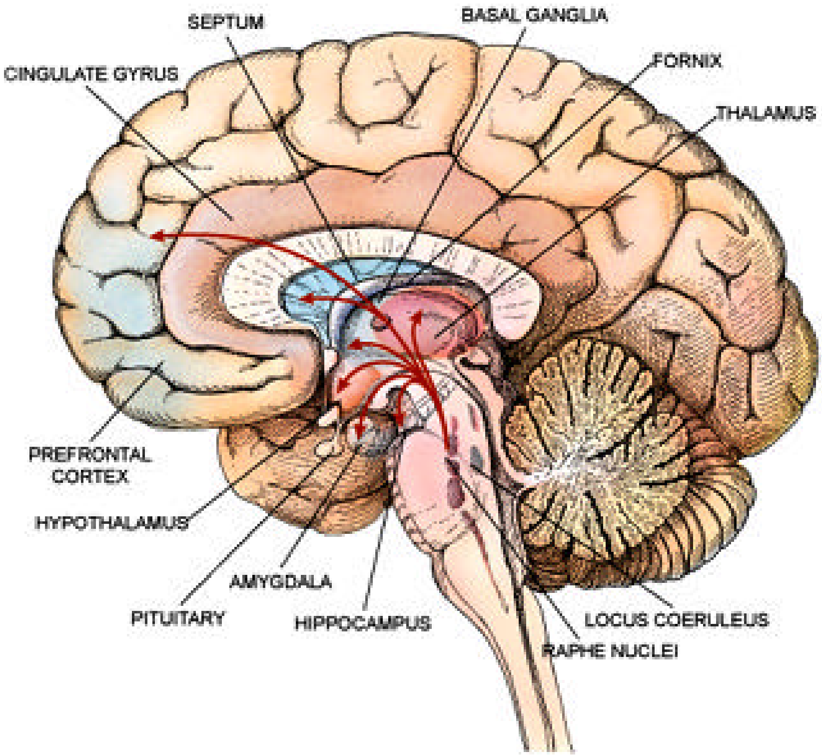


Figure 27
View of the Brain
Source:
http://www.sciam.com/1998/0698issue/0698nemeroffbox3.html
Scientific American
June 1998

movement, fine muscle movement, and balance (via the vestibular system of the inner ear). To help maintain a proper response it receives input from muscles and joints, the eyes and ears, skin, and other sensory organs.

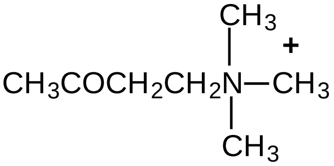
The **medulla** is the ultimate center for regulating vegetative functions such as respiration, swallowing, heart rate, and blood pressure. Related to respiration are the coughing and vomiting reflexes also associated with this part of the brain. The medulla is a type of crossroads where various neuron bundles go into and out of the brain. The **reticular activating system or RAS**, a part of the diffuse group of neurons known as the reticular formation,

connects the various portions of the medulla and extends upward into other areas of the brain. The RAS is responsible for maintaining a state of consciousness by arousing the cortex. It should be noted that direct stimulation of the cortex will not arouse the brain. Stimulation of the RAS will trigger cortical arousal. Drugs such as the amphetamines either mimic norephinephrine or cause its release in the RAS and are classified as stimulants. Conversely, depression of the RAS, as with general anesthetic drugs and barbiturates, leads to unconsciousness.

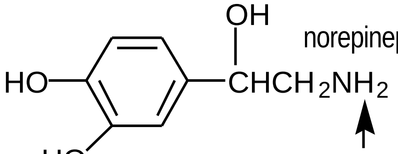
B. Neurotransmitters In The CNS

More than 60 chemical entities have been putatively identified as CNS neurotransmitters. Some of them, such as acetylcholine and norepinephrine, we have seen before in the peripheral nervous system. Because of the complexity of the brain you would expect more complex active molecules and the responses they elicit would be comparatively more complex. CNS neurotransmitters are often classified into structural types such as catecholamines (norephinephrine), biogenic amines (serotonin), quaternary ammonium compounds (acetylcholine), polypeptides/proteins (endorphins), and amino acids/amino acid analogues (gamma amino butyric acid). The activities of these neurotransmitters may be stimulatory or inhibitory (modulatory) depending upon the location of the neuron and the function of the area of the brain in which it is located. The continuation of the action potential in the postsynaptic membrane is usually brought about through the intervention of second messengers such as cAMP and DAG (diacylglycerol). The stimulation of the neurotransmitter is terminated in the ways discussed in the section on the peripheral nervous system. Amino acids and their analogs and the polypeptide agents are often secreted and/or taken up by active transport processes and consumed by neuronal uptake and enzymatic degradation.

There are a host of receptors for each neurotransmitter with varying types of responses. For example, the four subtypes of adrenergic receptors which we saw in the PNS are also present in the CNS (α_1, α_2, β_1, β_2). Cholinergic receptors come in two flavors, M_1 and M_2, while dopaminergic receptors show an elevation of cAMP levels upon stimulation of D_1 types and a decrease in cAMP after D_2 stimulation. Opiate receptors (μ,μ_1, δ, κ, σ)are the subject of intensive study as are 5-HT receptors. Drawn below are a few of the known CNS neurotransmitters and their activities as postulated.

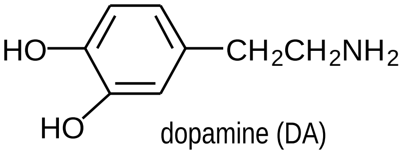


acetylcholine (Ach)



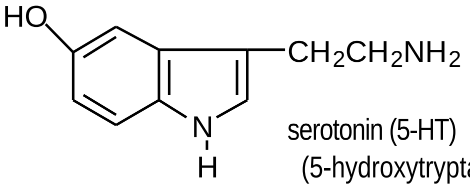
norepinephrine (NE)

(CH_3) epinephrine



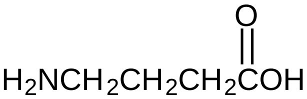
dopamine (DA)

found in limbic system and nigrostriatal pathways



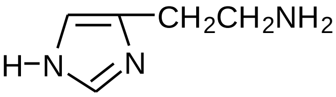
serotonin (5-HT)
(5-hydroxytryptamine)

usually inhibitory - cAMP mediation



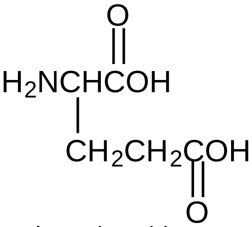
γ-aminobutyric acid
(GABA)

its inhibitory action involves increasing membrane permeability to Cl^- thereby hyperpolarizing it



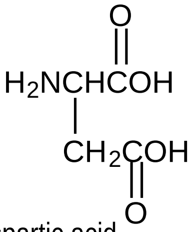
histamine

H_1 and H_2 receptors

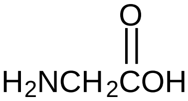


glutamic acid

stimulatory



aspartic acid
stimulatory



glycine

its inhibitory action is found in the brain stem and spinal column where it acts as a "brake" on motor neurons

Enkephalins
(natural opiates)

Tyr~Gly~Gly~Phe~Met
Met-enkephalin

Tyr~Gly~Gly~Phe~Leu
Leu-enkephalin

Glutamic acid has recently been implicated as a likely cause of excitatory neural degradation in the CNS. It, as well as other endogenous and exogenous agents, stimulate what is known as the NMDA receptor and somehow cause destruction of nerve cells.

More complex polypeptides, many of which also exhibit hormonal activity, have been implicated in the functioning of the CNS. Neurotensin, angiotensin II, oxytocin, vasopressin, somatostatin, thyrotropin- releasing

hormone, luteinizing-hormone-releasing hormone, adrenocorticotropic hormone, and other hypothalmic and pituitary products are among these.

C. Disease States

There are many conditions which affect the CNS system from headaches to homicidal mania. We will start with some "diseases" which have come to the attention of the public in recent years. These diseases most often affect our aging population - Parkinson's and Alzheimer's. The first is an example of a condition whose primary site of malfunction is known and which can be treated to a limited extent resulting in the prolongation of life and enhancement of the quality of that extendend lifetime. The latter disease is a cause of frustration and familial hardship not only because we are just beginning to get an idea about what is happening in the brain but also because there are no effective longterm treatments to prevent the prolonged deterioration of the mind ending in death.

1. Parkinson's Disease (PD) was first described in 1817 by the English physician, James Parkinson. Its victims suffer progressive muscular malfunction centered in dopamine(DA) and acetylcholine(Ach) mediated neurons in the substantia nigra and corpus striatum of the brain which control fine skeletal movement. In these areas dopamine modulates the muscle-stimulatory and modulatory actions of acetylcholine and GABA, respectively. The destruction eventually reaches 80-90% and the symptoms of the disease appear due to the unmodulated and excessive stimulation of motor neurons. The condition can frequently start with resting hand tremors and involuntary mouth and finger movement, progressing to a shuffling gait and poverty of movement (bradykinesia), eventually leading to muscle rigidity and difficulty in initiating movement (akinesia). Victims have a tendency to fall forwards or backwards and cannot catch themselves. 50% will also develop dementia.

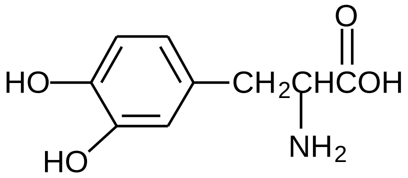
The etiology of Parkinson's disease is still very uncertain. It may be idiopathic, that is, of unknown origin, or secondary to trauma or drug treatment. Reports of the number of victims in the United States ranges from 400,000 to 1 million. About 50,000 new cases are diagnosed each year, mostly in those in late middle age, about 55. About 1% of senior citizens over 65, more men than women, have PD. At one time it was thought that Parkinson's was a condition of the 20th century but medical historians now believe that it has existed throughout the history of mankind. The observations of Leonardo DeVinci corroborate this belief in that he described the paucity of movement and tremors in victims during the 15th century. Many of the symptoms were probably attributed to old age.

It is known that secondary PD can be the result of damage to the brain by exogenous trauma or agents. For example Mn produces parkinsonian symptoms in exposed minors. Carbon monoxide, cyanide and carbon disulfide poisoning in industrial settings have ended up in conditions like PD.

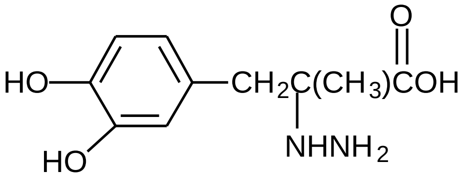
Drug treatment with certain agents such as specific types of neuroleptics and reserpine produce PD as a side effect.

As to what causes the idiopathic form, there are many theories. The encephalitis (***encephalitis lethargica***) pandemic which lasted from 1915 to 1920 produced parkinsonism which was different from that which has preceeded and followed those who might have been infected. Other theories involve autoimmune damage, genetic predisposition, other viral infection, even a fungus infection (***norcardia asteroides***- reported in May 1990). One of the difficulties in studying PD up to about ten years ago was the absence of an animal model. PD could not be induced in animals so investigators were left with only humans to study. In 1982 the work of Dr. J. William Langston was published in which he found a way to produce PD in primates. This has opened many doors for study. The work is summarized in an ASIDE.

Since the biochemical reason for idiopathic Parkinson's disease is a diminishing supply of dopamine, a precursor to norephinephrine, one course of therapy might be to provide exogenous dopamine. However, dopamine cannot pass the blood-brain barrier and is rapidly metabolized to inactive products. But the metabolite which gives rise to dopamine, that is, levo dihydroxyphenylalanine or **levoDOPA** (L-DOPA), can cross the blood-brain barrier. Once across it will be decarboxylated to DA in the basal ganglia. Since L-DOPA can also undergo similar metabolism before entering the brain, it is often administered with carbidopa, a DOPA decarboxylase inhibitor. Most patients require 400-1000 mg of L-DOPA given in divided doses 2 to 5 hours apart. The combination with carbidopa allows lower doses of L-DOPA. In July 1991 a slow release form of Sinement (Sinement CR®) was approved which contained 200 mg of L-DOPA and 50 mg of carbidopa compounded in a slow-dissolving resin.



levodopa (as Zn^{2+} or Cu^{2+} chelate (Larodopa®)



carbidopa

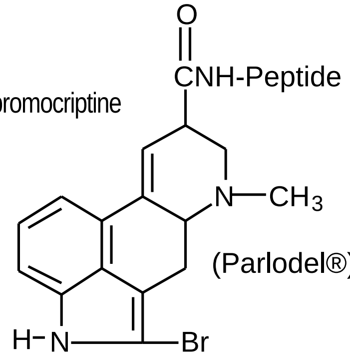
mixed in a 1 to 10 (L-DOPA/ carbidopa) ratio to produce Sinemet®

The results of levodopa treatment are dramatic. However, many of the side effects can be as bad or worse than the condition itself especially later in the course of the disease when doses are necessarily higher and taken with great frequency. These side effects include defects in voluntary movement (dyskinesias) such as grimace, tongue protrusion, head nodding and a rocking motion, GI upsets, flushing, mental aberrations (hallucinations and/or paranoia), orthostatic hypotension (the inability to rise from a lying or sitting position without a loss of blood pressure), and bradycardia. After 2 to 5 years of treatment more than 50% of PD patients experience and "on/off" effect, that is, fluctuations in response to their medication.

Experimentation has also been performed in tissue transplantation to replenish the cell lost in PD. Samples of a victim's own adrenal tissue have

been excised and placed into the area of the brain affected. Fetal brain tissue has also be transferred into patients. So far the results have been inconsistent with many patients showing significant improvement while others don't.

bromocriptine



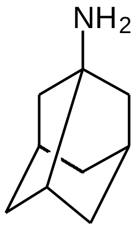
(Parlodel®)

Bromocriptine also inhibits the secretion of the hormone prolactin and can be used to treat some problems of male and female infertility and impotence as well as menstrual discomfort.

Bromocriptine (as the mesylate) has agonistic activity at brain D_1 receptors and antagonistic activity at D_2 receptors. It is used in the early stages of PD and to extend Sinemet treatment. Its side effects are similar to that of levodopa.

Amantidine is believed to enhance the release of DA from undestroyed neurons. Though not as effective as levodopa, it has fewer side effects.

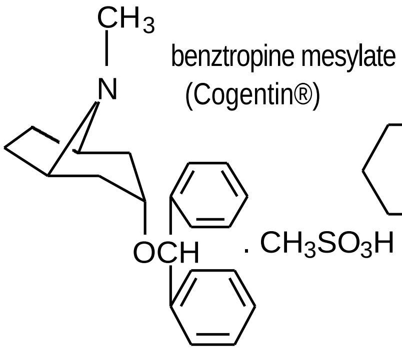
Other drugs such as some anticholinergics can be used in the early stages of parkinsonism to control the effects of excessive Ach stimulation. Benztropine, procyclidine, biperiden, and trihexyphenidyl are commonly used for this purpose. Specific antihistamines, such as diphenhydramine, also have such activity.



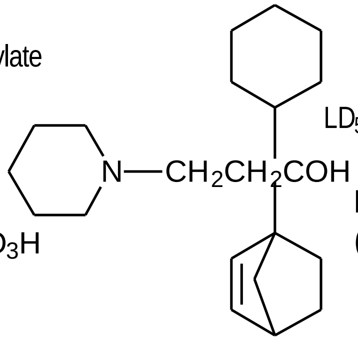
amantidine

(Symmetrel®)

Amantidine was originally developed to treat the early stages of infection by influenza A.



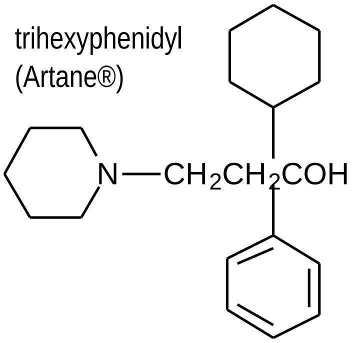
benztropine mesylate
(Cogentin®)



LD_{50} (mice): 545 mg/kg (oral)
56 mg/kg (i.v.)

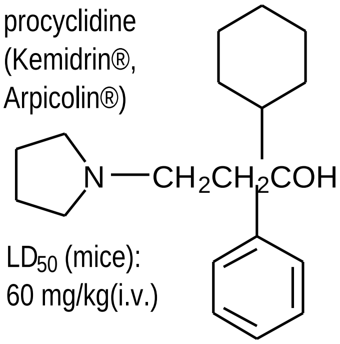
biperiden
(Akineton®, Akinophyl®)

trihexyphenidyl
(Artane®)



procyclidine
(Kemidrin®,
Arpicolin®)

LD_{50} (mice):
60 mg/kg(i.v.)



2. Alzheimer's Disease(AD) is a progressive and fatal deterioration of mental and physical abilities which currently affects about 3 to 4 million people in the United States, along with other forms of irreversible dementia, ranking fourth as the cause of death (100,000 per year). One in three American families is thought to have an Alzheimer's victim, real or potential. Alzheimer's disease costs the U.S. about $88 billion annually yet only $120 million of government funds is being used to study and combat the condition. The average American family will spend $25,000 per year to care for an AD patient but almost no public or private insurance reimbursements exist to help in this payment. A family must qualify for welfare before it can receive government aid from Medicaid.

Also referred to as senile dementia-Alzheimer's type, AD can begin as early as 30 years of age but it occurs most frequently in senior citizens and the frequency increases with age. 11.3% of those over 65 years old and 50% of those 85 or older are estimated to have AD (1991 figures). It strikes men and women alike and there seem to be some inheritable links. The risk for a theoretical lifespan of 100 yeras is 16% for those with no family history of AD and 24% for the first degree relatives of those with AD. As a hereditary disorder AD seems to be autosomal dominant with onset at 35 to 50 years of age. Diagnosis can be very difficult because the symptoms, especially in the early stages, such as loss of short-term memory, lack of concentration and depression, are common to other physiological conditions such as old age, stress, or stroke. However, the progression of AD is slow and unrelenting. Within three to ten years the victim will be devoid of reason, bedridden, incontinent, and unable to care for himself.

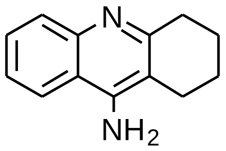
In order to absolutely confirm the presence of AD, the brain must be biopsied immediately after death. What is found is a general deterioration of brain neurons and the presence of excessive quantities of neurofibrillary tangles (twisted neuron bundles) and amorphous amyloid protein. In 1907 it was the neurofibrillary tangles which Alois Alzheimer first associated with the condition which bears his name. Today clinical psychiatric evaluations, PET brain scans for utilization of glucose as well as NMR are used to help in the diagnosis of AD. It is extremely difficult to differentiate AD from other types of

disorders in its early stages. However, current diagnostic procedures for cases presented are 80-85% correct.

The cause of AD is unknown although many hypotheses abound. The gene for one of the excessive amyloid proteins(b-type) has been associated with chromosome 21 at a point not far from a locus linked to some cases of familial Alzheimer's. Victims of Down's syndrome have an extra copy of chromosome 21 and usually fall prey to AD in their 40's should they live to that age. However no all AD victims have a 21 mutation. Other causative theories involve accumulations of aluminum in the brain or the presence of a slow virus or an infectious protein substance called a prion.

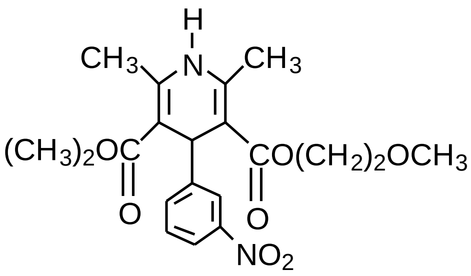
In June 1990 Dr. Peter Davies reported in JAMA that his research group had found a protein called Alzheimer's disease-associated protein (ADAP) in 86% of AD patients' brains with none found in control subjects. Abbott labs in early 1991 produced an ADAP detection kit to be used in postmortem investigations. They hope to develop similar tests to be used with cerebral spinal fluid.

During the summer of 1991 researchers announced the introduction of genes for amyloid protein into rats. They hope to be able to resolve the issue of whether amyloid is a cause or a result of Alzheimer's by observing whether the transgenic rats develop problems in processing information.



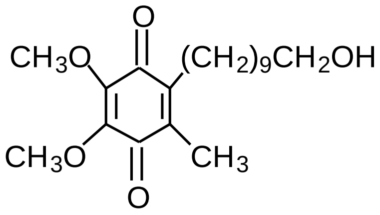
tetrahydroacridine or tacrine (Cognex®, Romotal®, THA) has anticholinesterase activity and is also used as an antidote for curare poisoning and as a respiratory stimulant

By and large the drug treatments for AD merely attempt to diminish the impact of the psychiatric symptoms. Major and minor tranquilizers and antidepressants, which we will be discussing shortly, can help in the management of AD victims in the early stages of the condition. Attempts have been made to treat apparent decreases in the amount of acetylcholine in the hippocampal area of the brain. This is due to lower choline acetyl transferase activity. Acetylcholinesterase inhibitors have been tested in an attempt to prolong the action of existing acetylcholine. Trials for what looked like a promising drug, tetrahydroaminoacridine (THA), were terminated in 1987 because of adverse side effects and apparent lack of efficacy. However, research with THA has continued due to some positive results with individuals. In July 1991 an FDA federal advisory panel recommended expanded use of the drug in carefully controlled clinical trials.



nimodipine (Nimotop®, Periplum®)
cerebral vasodilator

LD_{50}(mice): 3562 mg/kg (oral)
33 mg/kg (i.v.)
(rats): 6599 mg/kg (oral)
16 mg/kg (i.v.)



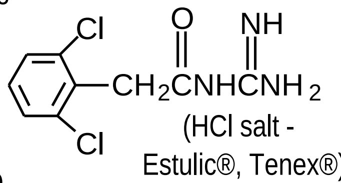
idebenone (Avan®) - a nootropic with some protective effects against cerebral ischemia

In the October 10, 1988 issue of **Chemical & Engineering News** there appeared a brief report from the Israel Institute for Biological Research about an acetylcholine analog, cis-2-methylspiro(1,3-oxathiolane-5,3')quinuclidine, which seems to be very specific for brain Ach receptors involved in AD. In the same report another drug in Phase I clinical trials was mentioned. Produced by Bristol-Myers it is meant to be used for a variety of cognitive memory disorders including AD.

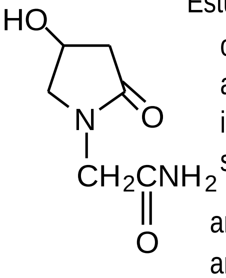
Experimentally areocoline (betel nuts) and the hormone vasopressin in a nasal spray have been used to enhance short term memory. A number of other drugs are being investigated most of which are already in use for other disorders.

It will take many years and increased funding to find the causes and treatments for Alzheimer's disease. The condition is extremely complex as it affects some of the least understood and most highly integrated areas of the brain. Many neurotransmitter systems are involved with the hippocampal acetylcholine system being the major one identified to this point in time. However, progress occurs in spurts and sputters. Perhaps we will see some effective treatment or preventive within our lifetimes.

guanfacine



(HCl salt - Estulic®, Tenex®)



oxiracetam
a nootropic - analog of iracetam with psycho-stimulatory activity

an antihypertensive and α_2-adrenergic agonist

LD_{50}(mice): 165 mg/kg (oral)

3. Huntington's Chorea (Disease) is an inherited condition of neurotransmitter hyperabundance as far as dopamine is concerned, with a decrease of GABA and Substance P. The result is a hyperkinetic state (chorea) with mental degeneration ending in dementia. The first signs of the disease do not usually appear until middle age (35-50). Walking becomes difficult as does swallowing. Psychiatric disturbances range from personality changes or apathy to manic-depression or schizophrenia. The dementia is

usually undetectable until it occurs full blown. This can cause serious psychological stress for children of Huntington's victims is the gene is autosomal dominant and 50% of the offspring of those afflicted will get the disease regardless of sex. The late Woody Guthrie, folksinger and composer ("This Land is Your Land"), died of Huntington's in 1946. His son, Arlo, has reached middle age.

The choreiform movements and behaviors can be only partially controlled by phenothiazines or butyrophenone neuroleptics.

Because of its rarity, it was difficult to study this fatal condition. However, within the last ten years a great deal has been learned about the genetics of the disease because of the discovery of a community in Venezuela rife with Huntington's. It seems that the dominance of the genetic link leaves the elders in families incapacitated with the younger members assuming the responsibility of care. This responsibility has been passed on from generation to generation resulting in the isolation of the group and resistance to outside genetic forces. Although no treatment exists for the condition, the study of the inheritable trait will eventually be coordinated with chemotherapy to provide a comprehensive plan for detection, prevention and aid.

D. Theories Of Mental Disease

The late nineteenth and early twentieth centuries were the era of Freud and the psychoanalytical theory of mental disorders. Everything from mild depression to full blown psychosis was believed to be rooted in abnormalities of the psyche which had to be treated with lengthy sessions on the couch. Although talking about one's problems is still part of most therapy programs, the medical profession has turned the corner into the modern world and realizes now that an imbalance of neurotransmitters can be the cause of many disorders of mood and even psychosis. In fact this is not a new idea in that even Freud considered biochemical imbalances before he framed his psychoanalytic theory. Overactivity as is observed with schizophrenia is believed to be due to an excess of norepinephrine and dopamine and/or a deficiency in serotonin (which acts as a "brake" to impulses) while depression involves a lower than normal amount of NE and DA. These biopsychological neurotransmitter hypotheses arose from the changes caused by administering, or the self-administration, of drugs which were found to alter specific neurotransmitter concentrations in the CNS. Humans have sought psychoactive relief from the stresses of daily living since the days of medicine men/women and magicians. But it wasn't until this century that scientific investigations were advanced enough to study the chemical changes occurring.

Psychiatry is still influenced by the theories of Freud and Jung while the biochemical evidence is slowly being accepted into the practice of mental healing. Because of the complexity of the CNS there are a host of diagnoses which can be applied to a set of symptoms.

Sigmund Freud

Carl Jung

Specific terms have been assigned to diagnostic criteria and are updated in the American Psychiatric Assoiciation's **Diagnostic and**

Statistical Manual of Mental Disorder IV (DSM-IV). We will be considering three broad categories of mental disease as we look at drugs which affect the CNS.

1. Psychosis is a state wherein the victim usually does not function within the "normal" tenets of society. S/he may have extremes of temperament from severe depression to euphoria without apparent cause. S/he may suffer from incorrect ideas, that is, illusions and delusions, or actually see and hear things which are not there (hallucinations). In other words, there is a noticeable absence of reality. Schizoid personalities are withddrawn, solitary, emotionally cold and distant. The fantasies they experience may be a way of coping. Misconceptions about the diagnosis and treatment of schizophrenia have led to the abandonment of thousands of mentally incapacitated to the streets without the care they need.

2. Affective disorders are those in which the person may be able to function but his/her responses are predetermined and they may be severely low or extremely high. A **unipolar depressive** may be without hope to the point of incapacitance and suicide. A **bipolar manic-depressive** cycles between abysmal lows and euphoric highs during which s/he is ennervated to the point of little sleep and is obsessed with activity. These conditions affect the famous as well as the poor. Some well-known manic-depressives include Ernest Hemingway, Abraham Lincoln, Vincent VanGogh, Handel, Robert Schumann, and Balzac. There have been further categorizations as disthymic disorders which are low level unipolar disturbances sometimes referred to as "personality disorder" and seasonal affective disorder (SAD), a depression occurring during the winter season of lengthening periods of darkness.

3. A large number of phobias and obsessions as well as anxieties are grouped under the heading of **neuroses**. A neurotic can still function in society but his/her patterns of behavior, though abnormal, can fit into or be compensated for by society. Recently attention has been focused on obsessive-compulsive behavior such as incessant hand-washing or ritual activity when entering or leaving rooms.

Keep in mind that the preceding terms are very broad in their scope and that there may be nuances which will further subdivide behaviors into more specific categories. There can also be a great deal of variation in any one person's personality and s/he may fit into more than one diagnosis or be misdiagnosed depending upon the person who is observing and the prevailing theory. In the middle of this century, for example, persons were diagnosed as schizophrenics who today would be said to be in the manic state of a bipolar affective disorder. Does this make a difference? Yes, it does in terms of the chemotherapeutic approach.

E. CNS Drugs Of Use And Abuse

1. Terms

There can be confusion as to the terms used when considering drugs affecting the CNS. This confusion can lead to misconceptions about the appropriate and inappropriate use of CNS drugs. Overall the terms used overlap to a great extent and lend themselves to several interpretations. The definitions cited below come from an article in **The New England Journal of Medicine** entitled "The Treatment of Cancer Pain" (**313** (2) 84-95, 1985. I have also added some language found in the **Merck Manual** in an attempt to clarify the issues involved.

Tolerance is the state in which escalating doses of a drug are needed to maintain an effect, such as pain relief (analgesia).

Physical dependence is a state of adaptation characterized by tolerance and the onset of acute symptoms and signs of withdrawal if the drug is stopped suddenly or an antagonist to the drug is administered (known as abstinence syndrome).

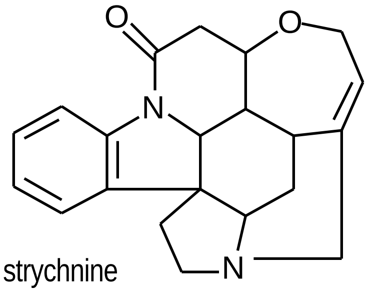
Psychological dependence is a factor separate from tolerance and physical dependence although it may involve both of those characteristics. Addiction carries with it a craving for the substance, satisfaction in using it, a desire to take it again in order to produce pleasure or avoid discomfort, and an overwhelming involvement in obtaining and using it.

Addiction may involve all or any combination of the terms above with the added perception of risk.

Added to these terms should be another - drug abuse. **Drug abuse**, also called chemical or substance abuse, has a definition dependent upon societal norms. It is the use of agents to the extent that they interfere with the health and normal social functioning of the individual (**Cocaine, Marijuana, Designer Drugs: Chemistry, Pharmacology, and Behavior** CRC Press 1989). This is not to be confused with **drug misuse** which can mean using a drug for purposes other than prescribed or in amounts other than prescribed. The AMA, when defining drug use versus abuse points out that it is crucial to consider the nature and amount of drug used, the situation in which it is used, the personality, experience and expections of the user and the prevailing attitudes of society towards the use of that drug.

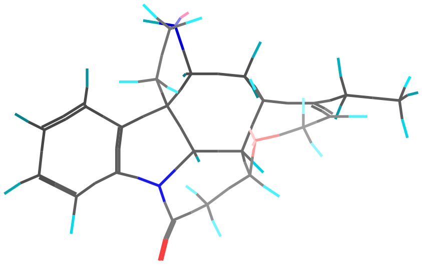
With these terms in mind we will begin a presentation of some of the principal drugs used therapeutically and recreationally to alter one's perception of the world.

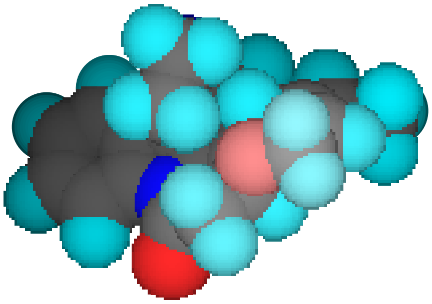
2. Stimulants



strychnine

LD_{50} (rats): 5 mg/kg (oral)





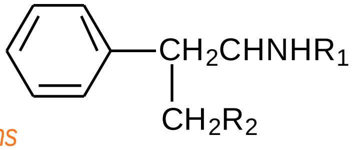
a. Strychnine is a alkaloid used as a poison for rats and other pests. It is isolated from the bark and seeds of the small tree, ***Strychnos nux vomica***, which can be found on the Indian subcontinent and in Sri Lanka (Ceylon). Its action is to block the inhibitory braking effects of glycine in the spinal motor neurons. As a result motor neurons will be fired without discrimination and convulsions will result. Death can occur from respiratory failure and asphyxiation or from exhaustion. The full body convulsions are preceded by stiffness and twitching in the muscles of the face and neck. In fact, the victim will have a gruesome grimace as a death mask due to the stretching of the mouth and face muscles. (Tetanus also produces a death grin but it is a beginning symptom rather than the last as seen in strychnine poisoning.) The antidote for such poisoning is an i.v. infusion of barbiturates and a quiet environment (so as not to stimulate the nervous system into a convulsive response). A related compound, brucine, can also be found in the bark of the nux vomica. Both alkaloids are extremely bitter to the taste.

b. Amphetamines were covered in the section on the peripheral nervous system since they will have stimulation at adrenergic synapses leading to an increase in heart rate and dilation of the pupils. Their anorexic effects are both peripheral and central. The CNS effects include increased alertness, a delayed need for sleep (REM sleep is decreased), and euphoria. Because of their structural similarity to norepinephrine and dopamine, these compounds stimulate NE and DA production as well as prevent their reuptake. They may also inhibit monoamine oxidases. The principal sites of action seem to be the limbic system and the RAS.

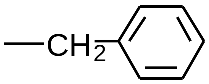
Amphetamines are definitely drugs which have been and are abused. Notice that the organic modifications in the fundamental amphetamine structure are to make the compound more lipophilic and so speed its delivery to the CNS as well as heighten the stimulant and euphoric effect. Therapeutic doses of amphetamines range from 5 to 10 mg for a maximum of 60 mg per day while abusive doses may be as high as 500-1000 mg every 2-3 hours. The side effects of abuse include tolerance, addiction, malnutrition, heart arrthymias, and

amphetamine psychosis, a state almost indistinguishable (except by testing) from paranoid schizophrenia. The psychotic aberrations may include vivid visual, tactile, auditory, and olfactory hallucinations. Withdrawal from heavy amphetamine use is not usually life-threatening. Rebound fatigue, depression and sleep, especially REM sleep, are experienced.

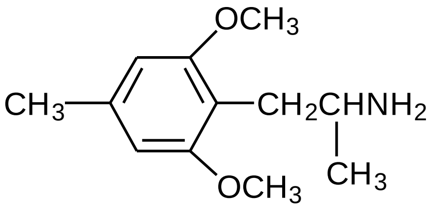
Methamphetamine is also known as "ice" and is a reoccuring drug abuse problem.



<u>amphetamines</u>

R_1	R_2	
H	H	d-isomer is dexedrine d,l racemic mixture is benzadrine (dexedrine is more CNS active)
CH_3	H	methamphetamine
CH_3		benzphetamine

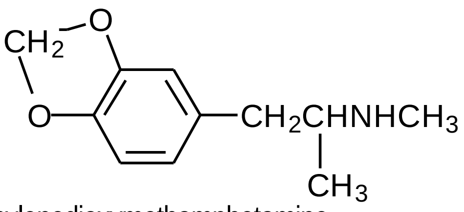
slang terms
benzedrine uppers, bennies, peaches
dexedrine dexies, oranges, hearts
meth, speed



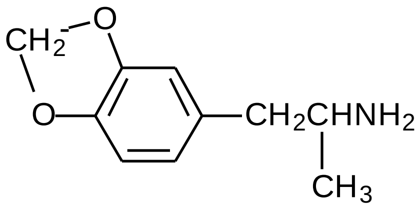
2,5-dimethoxy-4-methylamphetamine

STP, DOM

"Designer drugs" include amphetamines which have been modified for heightened psychoactive effects. The use of **MDMA** or Ecstasy has become popular over the past few years, especially after some psychiatrists attested to its beneficial use in alleviating anxiety and emotional trauma in their patients. **MDA**, a structural sister to MDMA, has been found to produce destruction of serotonergic neurons in rat brain.



methylenedioxymethamphetamine
MDMA
Ecstasy, Adam

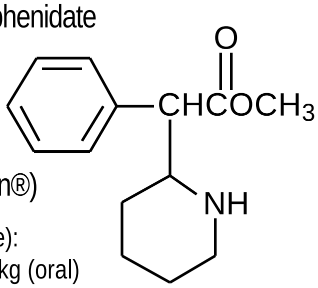


methylenedioxyamphetamine
MDA

Amphetamines are scheduled drugs which have a definite therapeutic use in the treatment of narcolepsy and attention deficit disorder (ADD) in children. Ritalin®, Dexedrine® and, to a lesser extent, Cylert® are used in the treatment of ADD. It is believed that ADD is caused by a deficiency of brain NE and DA which normally help a child to focus his or her attention on

schoolwork and productive behavior. The amphetamines supplement missing neurotransmitters.

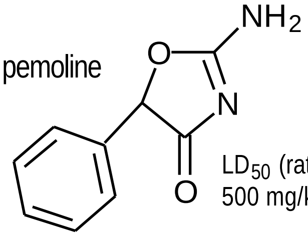
methylphenidate



(Ritalin®)

LD_{50} (mice):
190 mg/kg (oral)

pemoline



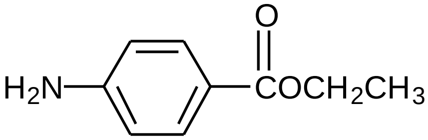
LD_{50} (rats):
500 mg/kg (oral)

(Cylert®, Deltamine®, Volital®, etc.)

c. **Local anesthetics** have a dual action. They block the sodium channels in pain-producing neurons and prevent NE and DA reuptake in adrenergic synapses, as well as perhaps 5-HT. The constant stimulation of DA receptors may lead to receptor "exhaustion" and an eventual lack of response as is seen in the effect of anhedonia - the inability to achieve pleasure - common to the abuse of cocaine.

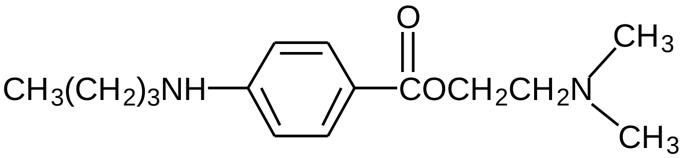
All of these drugs are metabolized by serine esterases. It should be noted, however, that babies cannot perform such hydrolysis reactions as readily and the materials will remain in the blood stream for four days.

The action of procaine is mainly as a local anesthetic and xylocaine does not stimulate the reward system of the brain the way that cocaine does.



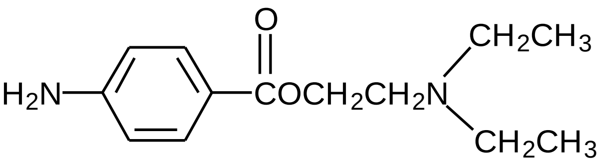
benzocaine

(Anbesol®, Cetacaine®, Chlorasceptic Lozenges®, Ayds®, Lanacane®, Solarcaine®, Vicks Throat Lozenges®)

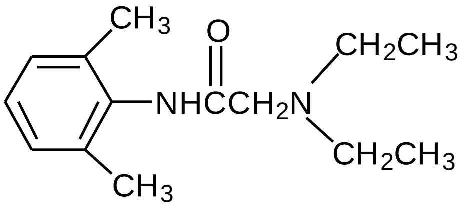


tetracaine

(Cetacaine®, Pontocaine®)

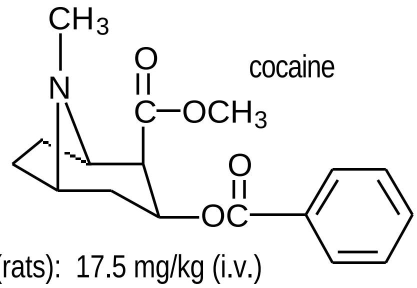


procaine (Novacain®)



xylocaine (Lidocaine®, Anesteon®)

The adverse effects of local anesthetic misuse or abuse include false sensory perceptions, tactile hallucinations, excitability leading to convulsions, and membrane deterioration.



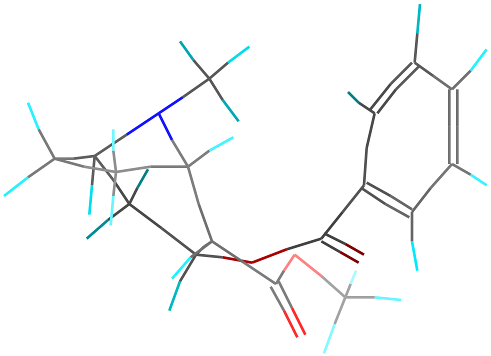
cocaine

LD_{50} (rats): 17.5 mg/kg (i.v.)

Uses: topical anesthetic for nose, eye, throat surgery

Cocaine is no stranger to us. We are very much aware of the abuse of this once helpful drug and the dangers not only to the psyche but also to the very existence of a body. The following is a chemical explanation of some of the features of cocaine preparation and use.

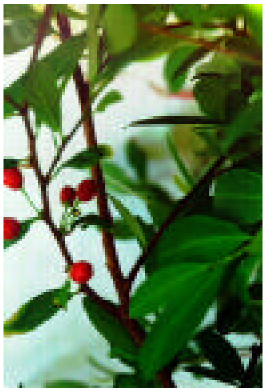
Cocaine (***coke, crack, rock, snow, dope, lady, gold dust***) is found in the leaves of ***Erthroxylon coca*** (about 2%) which grows at high elevations in the Andes Mountains (Columbia, Peru, Bolivia). The oval leaves can be picked four to five times per year, dried and chewed or extracted. The Incas venerated the coca bush and only priests and aristocrats were allowed to use it. However, the Spanish invasions of South America demystified the plant and presented an opportunity for trade with Europe. Coca was cultivated in Europe in the 1800s.



South American natives mix the leaves with ashes and pack the mixture between the cheek and gums. The absorption of cocaine under these conditions is slow and produces very little euphoria. The natives use this stimulant in order to survive the high altitudes and hard life they have to contend with.

The extraction process can be performed in acidic conditions which produces the cocaine as its hydrochloride salt. Alkaline conditions will produce a lipophilic "free base" which can be extracted with a nonpolar solvent like ether. The ether can be volatilized with heat and the free base smoked, snorted, injected, or eaten. The salt form cannot be smoked. Cocaine paste, not common in the U.S. is a kerosene extract which may contain 20-90% cocaine sulfate. Crack is the result of free-basing with baking soda (sodium bicarbonate). The residual fillers and other impurities

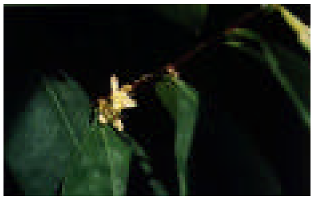
as well as the bicarbonate cause the cocaine to form chunks and crackle when burned. Several years ago the purity of street cocaine powder averaged 15%. Today crack will contain 80% of more cocaine.



The methods of cocaine administration include ingestion, inhalation of the powder (snorting) or smoke, topical, and intravenous injection. The intensity and duration of action are a function of the mode of administration. I.V. injection and smoking are the fastest ways to initiate the effects. The quicker the "rush" the shorter the duration of action. Snorting results in peak plasma concentrations of 150-200 ng/mL while smoking results in >900 ng/mL. A snorter's 3-5 cm "line" of cocaine will be about 20-30 mg of material.

The metabolism of cocaine leaves the person with an abnormal letdown, called "crashing". In an attempt to soften this severe depression cocaine is often compounded with heroin called a "speedball". This is common among those who started out as either heroin or cocaine abusers. The comedian John Belushi died from such a mixture.

As an adrenergic agonist the peripheral effects of cocaine include an increase in blood pressure and the induction of cardiac arrhythmias. Studies of sudden death in novice as well as experienced drug abusers found that cocaine causes vasoconstriction of the coronary arteries which seems to result from an enhancement of Ca^{2+} influx across myocardial membranes. However, remember that this class of drug affects other neurotransmitter systems. Cocaine inhbiits reuptake of NE and 5-HT as well as binds to the DA transporter. It increases catecholamine receptor sensitivity but does not seem to directly influence enkephalinergic receptors. In addition it also affects neurotransmission the H, Ach and phenylethylamine pathways. Activation of DA, NE or 5-HT neurons independently does not produce the euphoria associated with cocaine misuse. Euphoria seems to be related to simultaneous inteeraction between catecholamine and serotoninergic systems.



In an effort to transport cocaine into the country persons have become "body packers" who swallow balloons containing 85-90% cocaine. The average number of such bags of material which can be carried in this way is about 175. As you might imagine, should the bags break, the holder will be rapidly toxified. The progression of symptoms is extremely rapid: hyperthermia, hypertension, tachycardia and mydriasis, acute agitation and delirium, grand mal seizures, and finally respiratory arrest. Treatment for acute cocaine toxicity includes diazepam for convulsions, propanolol for arrhythmias, and chlorpromazine to calm general autonomic effects.

Those who abuse cocaine and other stimulants can also show psychotic effects which are not usually observed with marijuana,

depressants or psychomimetics: hyperactivity (head bobbing, repetitive acts), delusions of parasitosis (cocaine bugs), visual hallucinations (snow lights), tinkering, even aggressive and assaultive behavior.

The psychic damage and addictive force of cocaine has been duly reported over the last few years. Many questions still remain unanswered, such as, does everyone who uses it become addicted? Is there such an animal as an "addictive personality"? How does cocaine cause sudden death? Can this be avoided? Are there any drugs which can help to relieve cocaine addiction?

It is interesting to note that there may be susceptible personality types for substance abuse. For example, it has been estimated that around 10% of known cocaine abusers have attention deficit disorder. Sometimes the administration of ritalin will help in fighting the abuse.

Other drugs of the depressant, antianxiety, antipyschotic, and anticonvulsive types are being investigated as treatments for cocaine abuse. Those which have been or will be covered in this course include the heterocyclic antidepressants desipramine and imipramine, which diminish cocaine use and craving as well as improve the outcome in the first few months of treatment. Buprenorphine (depressant) may augment the reward system (it has been found to suppress self-administration of cocaine in monkeys). Lithium sometimes works for those who are clinically depressives. Carbamazapine, bromocriptine and mazindol are also used as well as fluphenthixol and buspirone.

Much of the information cited above came from the following two articles: **Science 246** 1376-1381 (12/15/89) and **Science 251** 1580-1586 (3/29/91)

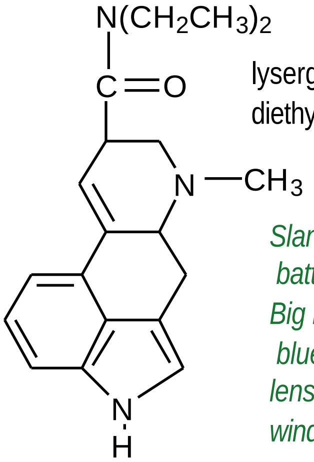
Some of the historical aspects relating to cocaine are very interesting. For example Sigmund Freud recommended cocaine to cure morphine addiction and the original concoction known as Coca Cola had "the real thing".

3. Psychomimetics

These CNS stimulants have sometimes been referred to as hallucinogens but are more appropriately referred to as psychomimetics because few, if any, cause an absence of reality, that is, a hallucination. Psychomimetics distort or heighten sensory input, produce dream-like states, and can be psychologically addictive. There is no consistent evidence that they can produce tolerance or physical dependence. This is not to imply that these drugs are innocuous. The distortions of reality can lead to panic, anxiety, lack of concentration, and psychotic states to say the least.

Besides NE and DA agonistic effects, the psychomimetics are also believed to impinge upon the serotonergic neurons. Serotonin stimulation can promote sleep as well as increase blood pressure and heart rate, produce tremors, constrict blood vessels, and cause pupil dilation just as

NE and DA can. Look at the structures of the molecules which follow and see how they may be chemical analogues of serotonin or the catecholamines.

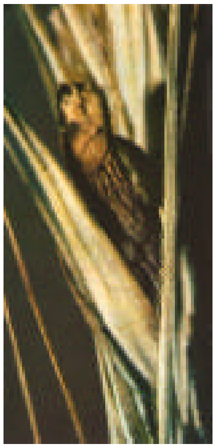


lysergic acid diethylamide (LSD)

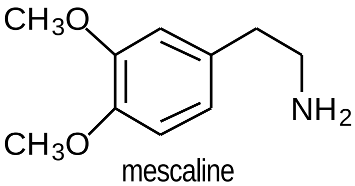
Slang terms: acid, barrels, battery acid,Berkeley blood, Big D, blotter acid, blue acid, blue microdot, chief, contact lens, HCP, sugar,sunshine, window pane, Zen

Psychoactive dose: 0.05 mg

a. **Lysergic acid diethylamide (LSD)** is a Schedule I drug, a semisynthetic ergot alkaloid whose parent compound can be isolated from rye fungus, ***Clariceps purpurea***, and morning glory seeds, ***Ipomoea violacea*** and ***Turbina corymbosa***. Recall the section on ergotamine. LSD affects both noradrenergic and serotonergic systems producing a distortion of sensory input, intoxication, and anxiety. Much of the LSD experience is influenced by the environment in which it is used. This is true of most psychoactive drugs. The ancient Aztecs worshipped and used morning glory seeds and indeed, they are still used to some extent by natives in the hinterlands of Mexico. There is evidence for the theory that convicted "witches" as well as the local population in the Massachusetts Bay Colonies were poisoned by ergot. This hypothesis is supported by historical accounts of ergotism in European communities during and after the Middle Ages. Flashbacks are an extremely disturbing aftereffect of use. LSD was first synthesized in 1943 by Albert Hoffman and was tested at one time by the Armed Forces as a possible incapacitating psychic agent. Its unpredictability and aftereffects made it of dubious worth.



b. **Mescaline** is the psychoactive ingredient in peyote, ***Lophophora williamsii***. A Schedule I substance, the peyote cactus contains about 30 psychoactive constituents of two main structural types, phenylalkylamines and isoquinolines. Mescaline is believed to be responsible for the "color visions" experienced by users. The spineless crown of the cactus is cut from the room and dried into a "mescal button". The dried heads retain their potency over time and can be stored for use later. The active psycholgenic agents are not volatile.



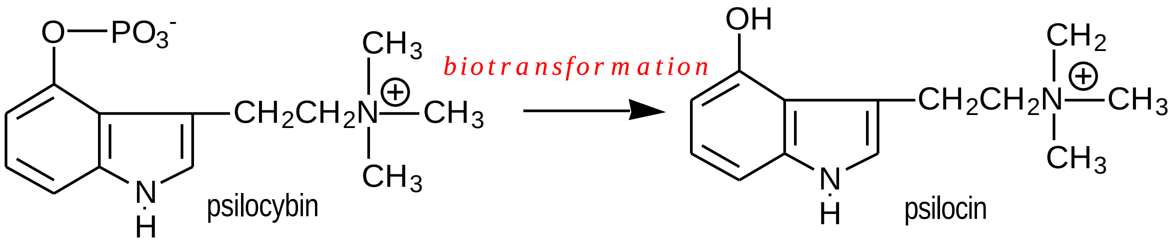
mescaline

bad seed, cactus, chief, peyote, pink wedge, white light

Peyote is a central element of the religious rituals of the Native American Church which is practiced by more than forty American Indian tribes in the U.S. and Canada, among them the Kiowa and Comanche. A 1918 law forbade the use of peyote for any reason but this law was declared unconstitutional in 1964 for the practitioners of the Native American Church.

Mescaline is not metabolized to any great extent and has a duration of action from 6 to 10 hours. The effective dose is 0.2-0.4 grams taken orally. Intoxication proceeds through two phases: the first produces a feeling of contentment and muscular sluggishness while the second involves a shift of attention from external stimuli to a more introspective, meditative state. It is important to realize that the context in which this psyhoactive substance is used has a direct bearing on the mental state achieved and that the peyote is considered a sacrament. In that setting the drug is neither being abused nor misused.

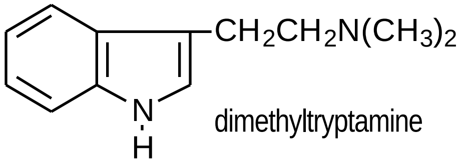
c. Psilocybin and psilocin are also Schedule I drugs. They are found in mushrooms of the genus ***Psilocybe***. Although many species of Psilocybe can be found thoughout the Americas and in some parts of Europe and Asia, the most psychoactive seem to be from southern Mexico where they are still used in rituals. Psilocybin and its biotransformation product produce visual and auditory hallucinations, cause dilation of the pupils, make concentration difficult, result in muscular limpness which can result in a fatal respiratory depression, and can produce a paranoid schizophrenia state. Tolerance does develop over time although the possibility of physical and psychological dependency seem low.



exotic mushroom, God's flesh

Cross tolerance has been demonstrated between LSD and mescaline, psilocybin, and psilocin. There seems to be no cross tolerance between LSD and marijuana or amphetamine. These observations are indicative of the structural similarites of the compounds.

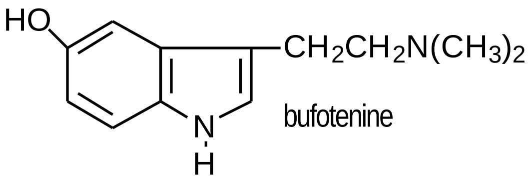
d. Other tryptamine derivatives



dimethyltryptamine

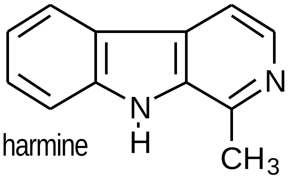
Dimethyltryptamine (DMT) is a synthetic psychomimetic with a very short duration of action. This has given it the slang name of "businessman's trip" since it can be taken over the lunch hour. The diethyl derivative is also effective.

Bufotenine is also a tryptamine derivative isolated from species of the ***Anadenantera***, a pod-producing plant found in South America and the seeds from the pod can be toasted and ground into a snuff or smoked.



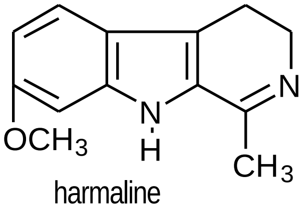
bufotenine

Bufotenine can also be exuded from the skin of the cane toad (hence the prefix bufo-). This is a red or green toad which was mistakenly purported to be the object of "toad licking" in order to become intoxicated. The reports of such activity were highly exaggerated. Licking the skin of the cane toad has made the lickers extremely ill. In fact in South America indigenous tribes used the material as an arrow poison.



harmine

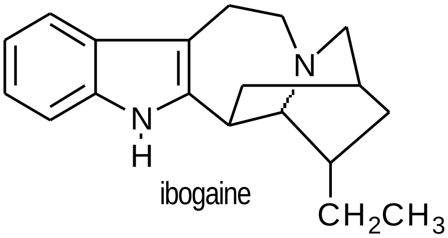
Harmine and **harmaline**, found in various species of ***Banisteriopsis*** found in South America, are also made into snuffs and used in religious rituals.



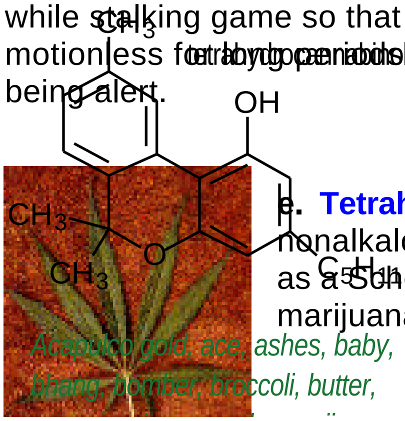
harmaline

Ibogaine can be isolated from the root of a shrub, ***Tabernanthe iboga***, found in West Central Africa (Gabon and the Congo). Used in the magical and religious cult activities of the Bwiti cult, an extract produces feelings of levitation, colorful visions and

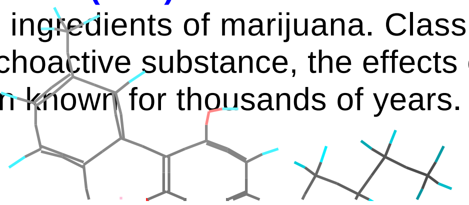
expands the concept of time. Practioners use the drug in initiation rites and to make contact with ancestral spirits. Natives are reported to take it while stalking game so that they can remain motionless for long periods of time while still being alert.



ibogaine

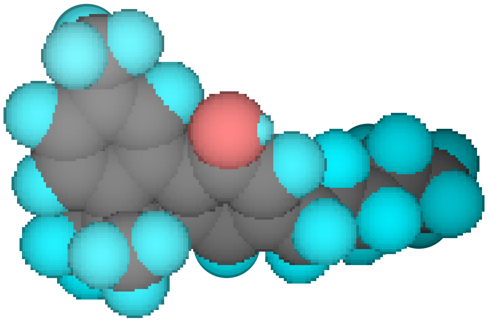


e. Tetrahydrocannabinol (THC) is the one of the nonalkaloidal active ingredients of marijuana. Classified as a Schedule I psychoactive substance, the effects of marijuana have been known for thousands of years. The



Cannabis plant from which it is harvested is found in most parts of the world. The plant has been used medicinally and for its hemp and food value throughout recorded history. The highest concentration of THC can be found in the resin of the pistillate, although the dried leaves are more accessible even though they are less potent. The effects of Cannabis vary greatly depending upon the type of plant used, method of preparation, dose, mode of administration, personality of the user and the situation in which it is used. Dream-like states, altered perceptions of time, euphoria, exitement, depression, hallucinations, and occasionally aggression may occur.

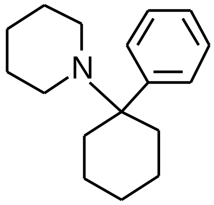
THC is optically active and the levorotatory form is 10 to 15 times more potent than the dextrorotatory. The therapeutic index (TI) has been reported to be 40,000. Due to its lipophilicity, THC crosses the placental membrane and is stored in fat deposits in the body. In fact its pattern of appearance in the plasma is bimodal, that is, it shows up almost immediately after use to a certain extent and then reappears over a period of anywhere from 14 to 30 days as it is gradually released from fat stores and metabolized. The



metabolites may therefore identified in the urine up to one month after use. Methods of detection are sensitive up to 10^{-7} moles in 20 mL of body fluid.

The Schedule I designation of marijuana has been disputed over the past 15 or more years. Some physicians would like to see it as a Schedule II drug so that it could be used therapeutically in the treatment of the nausea, vomiting and anxiety caused by cancer chemotherapy and as an antiglaucoma agent (lowers intraocular pressure). It should be noted that the neuroleptic prochlorperazine is an effective antinausea drug which can be used without producing the psychoactive effects of marijuana.

There is a putative receptor for THC and it is currently under investigation.



PCP

phencyclidine 1-(1-phenylcyclohexyl) piperadine

angeldust, flying saucers, hair, horse, tranquilizeer, mist, peace pill, dust, superweed, kools, wack, lovelies

f. Phencyclidine (PCP) was originally synthesized in 1950 as a possible human and veterinary anesthetic and was thought to have few, if any side effects. Experimentation showed that 5-10 mg in a human resulted a dramatic loss of sensitivity to pain. However, its use was abandoned because of

postoperative thought disturbances and agitation. Low doses produce a stae of agitation, excitement, gross uncoordination, a blank stare, catatonia, flushing, profuse perspiration, and rapid involuntary vibration of the eyeballs (nystagmus). Moderate doses result in a stupor or coma with the eyes remaining open, pupils in a fixed mid-position, vomiting, hypersalivation, shivering, and fever. Then high doses produce a prolonged coma with eyes closed, hypertension and convulsions. Treatment involves acidifying the urine (why?).

The biotransformation of PCP is oxidative and slow. It is believed that the oxidized product may be involved in covalent interactions with proteins resulting in the blocking of potassium channels and premature release of neurotransmitters. Another mechanistic possibility relates its structural similarities to NE, Ach and 5-HT. They have led researchers to believe that its mechanism of action may involve impaired NE reuptake and/or MAO inhibition and/or Achase inhibition and/or acting as an antimuscarinic.

Classified as Schedule I, PCP is an extremely dangerous substance which periodically visits the clandestine drug scene. There it can be found as a water soluble white powder or tablets which can be dissolved so that cigarettes can be dipped into the solution and smoked.

PCP and its "designer" analogs (TCP, PCE, PCPY, PCC, ketamine) pose a particular threat to law enforecement officers and cleanup crews who can rapidly become intoxicated. In fact, this is a problem with any of the illegal drugs. As you might imagine, disposal carries its own risks.

4. CNS Depressants

There are a number of different structural types of CNS depressants. Their modes of action are putative, that is, they are generally unknown but we have some indirect evidence of mechanisms. Much of what is known arises from their observed effects and not necessarily from direct experimental evidence. In fact, some of the drugs we will discuss, such as the opiates, have been around for millenia, long before any biochemical theories concerning their actions were conjectured. In the case of the opiates, it was their known effects which led researchers like Solomon Snyder and Candace Pert to theorize about the existence of natural (endogenous) opiate neurotransmitters, which they eventually found in the lab. The PBS NOVA segment entitled "The Keys to Paradise" and available in the Cal Poly libray describes the discovery of endogenous opiates.

A key structural feature to all CNS depressants is their lipophilicity. As we proceed through the structural categories, try to correlate the duration of action and potency of the drugs in question with their lipophilic character wherever possible.

a. Minor Tranquilizers

The minor tranquilizers include a variety of compounds used to treat anxiety, tension, irritability, and stress. This is in contrast to the major tranquilizers which are found in the treatment of overt mental disease such as schizophrenia and manic-depression. The terms minor and major should not be construed to refer to the relative toxicity of these drugs. All are CNS depressants and this pharmacological class are potentially lethal.

Barbiturates are referred to as sedative-hypnotics. These drugs will induce sleep which can lead to even deeper sedation (hypnosis) and can cause a fatal depression of the RAS affecting the respiratory system. The sleep which is encountered does not have the normal cycles of slow wave and rapid eye movement activity, so it is not always restful. However, these agents prove to be useful in anesthesia for both short and longer durations of time. Many of you may have been given thiopental prior to wisdom tooth extraction. Thiopental "wears off" quickly and so the actual anesthetic for the time of the extraction is usually nitrous oxide.

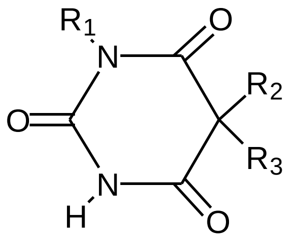
During the 1950s and 1960s barbiturates were fairly routinely prescribed for the treatment of anxiety. Their use proved to be addictive, led to tolerance and physical dependence. The physical dependence is life-threatening in that precipitate withdrawal from a heavy, chronic use of barbiturates can lead to death. It is also known that even though tolerance may be experienced the TI for barbiturates remains the same and a person who is consuming large doses of these drugs may be a few milligrams away from death. In the elderly barbiturates can cause paradoxical excitement, suppress REM sleep, cause rebound nightmares and insomnia.

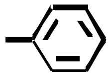
It is believed that the site of action for barbiturates is the receptor for the inhibitory neurotransmitter GABA, gamma amino butyric acid. GABA acts to modulate incoming excitatory signals by increasing the postsynaptic neuron's permeability to chloride ion which will hyperpolarize the membrane. This compounds may either enhance GABA binding so that it is more effective or they may have GABA activity themselves and so be additive to normal GABA activity.

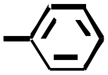
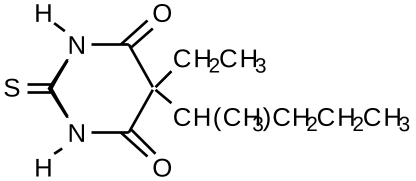
Although barbiturates are not currently used for the treatment of anxiety because of the abuse and misuse potential, they are prescribed for epilepsy and other convulsive conditions as well as for the treatment of colitis and tension headache. Some combination drugs for congestion also contain barbiturates.

The general structure of barbiturates.

LA-long acting
IA-intermediate acting
SA-short acting



R1	R2	R3	Generic Name/Duration	Tradenames
-H	$-CH_2CH_3$		phenobarbital (LA)	Broncholixir, Sulfoton, etc.

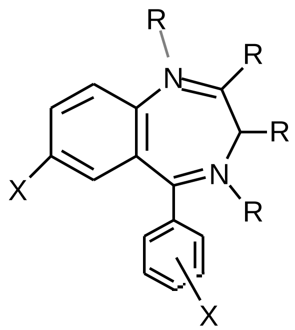
-H	$-CH_2CH_3$	$-CH(CH_2)_2CH_3$ CH_3	pentobarbital (SA)	Nembutal, Webcon, Antinausea Supprettes
-H	$-CH_2CH=CH_2$	$-CH(CH_2)_2CH_3$ CH_3	secobarbital (SA)	Seconal, Tuinal
-H	$-CH_2CH=CH_2$	$-CH_2CH(CH_3)_2$	butalbital (IA)	Amaphen, Phrenilin, Repan, etc.
-H	$-CH_2CH=CH_2$	$-CH(CH_3)_2$	aprobarbital	Alurate
-H	$-CH_2CH_3$	$-CH(CH_2)_2CH_3$ CH_3	butabarbital (IA)	Pyridium Plus, Quibron Plus
$-CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	metharbital (LA)	Gemonil
$-CH_3$	$-CH_2CH_3$		mephobarbital	Mebaral, Isonal
			thiopental	Pentothal, Nesonal, Intraval, Thiothanl, etc.

Treatment of colitis: Donnatal® and Kinesed® - mixture of phenobarbital, scopolamine, hyoscyamine, atropine (what a combination!)

Tension headache remedy: Buff-A-Comp® - butalbital

Bronchodilation/mucolytic agent: Mudrane® - mixture of phenobarbital, aminophylline, ephedrine

Phenobarbital, mephobarbital and metarbital are the only oral anticonvulsants which are effective at sub-hypnotic levels. Many barbiturates are classified as Schedule II, III, or IV due to their high potential for overdose and dependence. Abrupt withdrawal may cause seizures, restlessness, trembling, and insomnia and may be fatal. Phenobarbital is used as an anticonvulsant for the treatment of epilepsy and in some combination medications for the relief of irritable bowel syndrome.

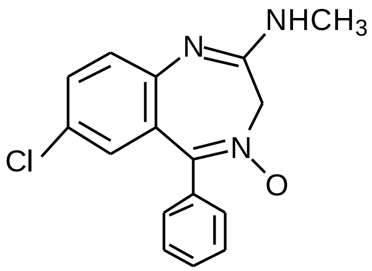


General Structure for Benzodiazepines

Benzodiazepines are also known as minor tranquilizers. Not as potent or dangerous as barbiturates, the benzodiazepines do find use as antianxiety drugs because of their safety and efficacy. In higher doses they will also induce sleep. All benzodiazepines cause a dose-

related CNS depressant effect varying from mild impairment of task performance to hypnosis.

Anxiety is a condition of stress or fear for no apparent reason. It is produced by excessive stress which, as we could conjecture, would upset the balance of neurotransmitters in the CNS and PNS. Besides the psychological effects, a person might also experience shaking, heart palpitations, GI upset, breathlessness, and headache.

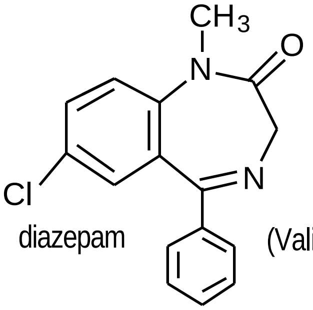


chlordiazepoxide (Librium®, Libritabs®, Menrium®)

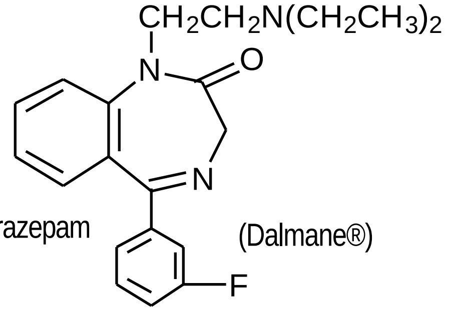
In contrast to the barbiturates, benzodiazepines do not produce an anesthetized state. They may be used as a preanesthetic in order to lessen anxiety, to be followed by a barbiturate and then the general anesthesia gas such as halothane.

Tolerance, physical dependence and addiction are possible with the benzodiazepines but less likely to occur than with barbiturates. In general this class of compounds does not cause induction. The potential for suicide is also lessened with these compounds. It has been estimated that physical dependence occurs in one person out of five million. Withdrawal symptoms are real but usually not life-threatening (fatigue due to REM rebound, dizziness, CNS disturbances). In general, benzodiazepines do not cause induction.

Similar to the barbiturates, the mode of action of benzodiazepines is thought to involve GABA receptors. They may enhance GABA binding and/or activate a feedback mechanism which curtails GABA release. Both mechanisms would result in making the nervous system more dependent upon continued drug activity.



diazepam (Valium®)

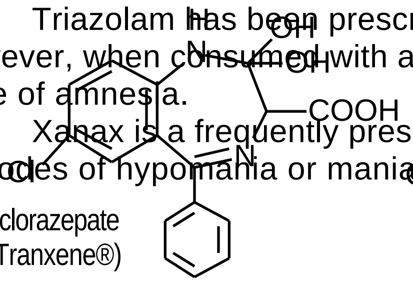


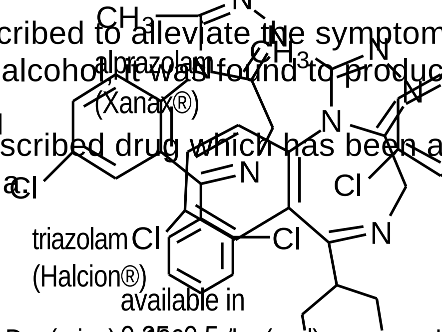
flurazepam (Dalmane®)

LD_{50} (mice): 870 mg/kg (oral)
(rats): 1232 mg/kg
(rabbits): 568 mg/kg

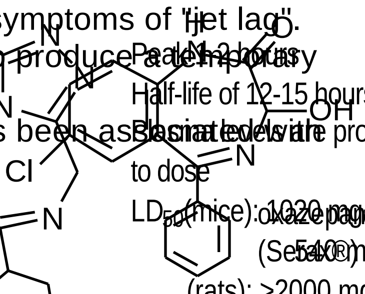
Triazolam has been prescribed to alleviate the symptoms of "jet lag". However, when consumed with alcohol, it was found to produce a temporary state of amnesia.

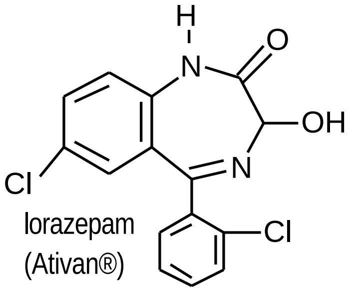
Xanax is a frequently prescribed drug which has been associated with episodes of hypomania or mania.


clorazepate (Tranxene®)


triazolam (Halcion®)
available in

alprazolam (Xanax®)
Peak 1-2 hours
Half-life of 12-15 hours
Plasma levels are proportional to dose
LD_{50} (mice): 1020 mg/kg (oral)
540 mg/kg (i.p.)
(rats): >2000 mg/kg (oral)


oxazepam (Serax®)

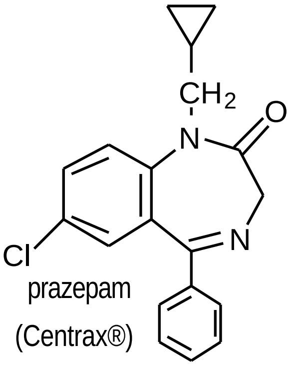


lorazepam (Ativan®)

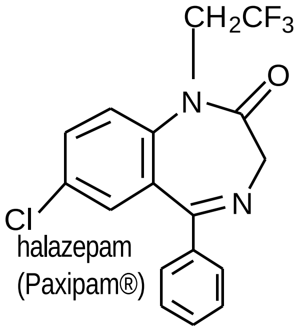
LD50 (mice): >3x10[3] mg/kg

(rats): >5x10[3]

(dogs):>2x10[3]



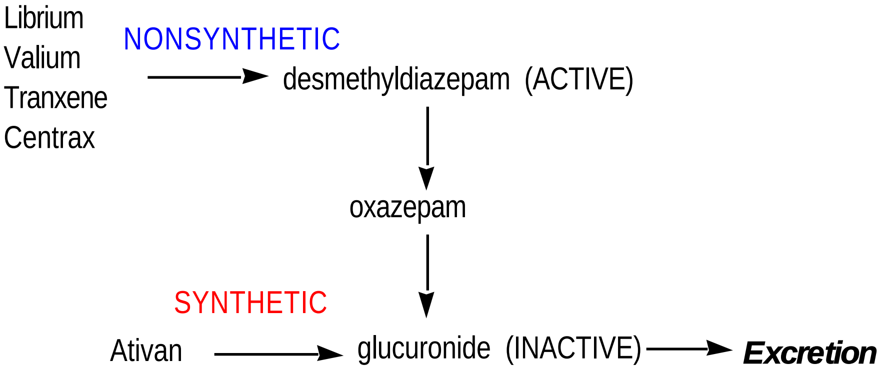
prazepam (Centrax®)



halazepam (Paxipam®)

LD50(mice): >4000 mg/kg (oral)

Although the benzodiazepines are structurally related, they do not participate in the same routes of biotransformation. Therefore the drug prescribed may depend upon the patient's diet or other prescription drugs being taken.



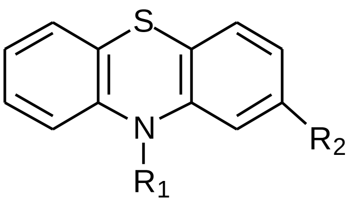
b. Major tranquilizers

These drugs are used to treat serious mental disease such as the manic phase of manic depression, organic pyschosis, paranoia, or schizophrenia. They are also called antipsychotics or neuroleptics. Because episodes of mental disease can undergo spontaneous remission and due to the potency and undesirable side effects of these drugs,they are carefully prescribed and their effects closely monitored.

Theories about the causes of mental disease are just that - theories. Various areas of the brain are involved in integrating "normal" behaviour patterns. DA, NE and 5-HT are believed to be key neurotransmitters in these systems. An overactivity in dopaminergic or adrenergic neurons may bring on a hyperagitated state. The neuroleptics were introduced into treatment in

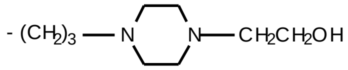
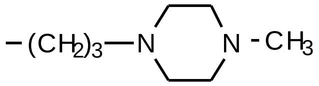
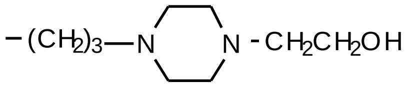
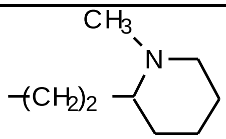
the 1950s and caused a revolution in psychiatric care. The major tranquilizers are thought to act as DA antagonists. Their nonproductive blocking of DA_2 receptors occurs in the limbic system where the effect will be an emotional "quietening", in the brainstem where emotional arousal will be quelled, in the basal ganglia , and in the hypothalamus which indirectly affects hormonal activity in the body. Some antipsychotics also block NE receptors. The effects of these actions are not entirely beneficial to the patient. Because of these antagonistic actions the side effects of treatment can be parkinsonism, tardive dyskinesia (jerking movements of the mouth, tongue and extremities), and restlessness. Sudden withdrawal of antipsychotic medications can cause GI upsets, sweating, headache, and anxiety.

Phenothiazines are antipsyhotics which may also be used as antiemetics because of their actions on the brainstem vomiting centers. In addition to the negative side effects observed with any antipsychotic, patients under phenothiazine regime may have a shuffling gait ("Thorazine shuffle"), weight gain, dizziness, lethargy, and photosensitivity.



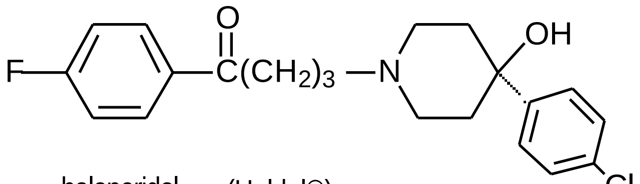
general structure of the phenothiazines

The use of phenothiazines for acute schizophrenia is preferred because of several advantages they afford. They cause little sedation and the patient is therefore easily aroused. Intellectual abilities remain intact as well as avoidance behavior. This is in contrast to the barbiturates whose hypnotic properties make the patient quite unaware of what is going on around her/him.

R_1	R_2	Generic Name	Tradenames
$-(CH_2)_3N(CH_3)_2$	-Cl	chlorpromazine	Thorazine
	-Cl	perphenazine	Etrafon, Triavil, Triafon
	-Cl	prochlorperazine	Compazine, Meterazine
	$-CF_3$	fluphenazine	Elinol, Pacinol, Tensofin, Vespazine, etc.
	$-SCH_3$	thioridazine	Mellaril, Sonapax, etc .
$-CH_2CH(CH_3)N(CH_3)_2$	-H	promethazine	Phenergan - used to potentiate codeine

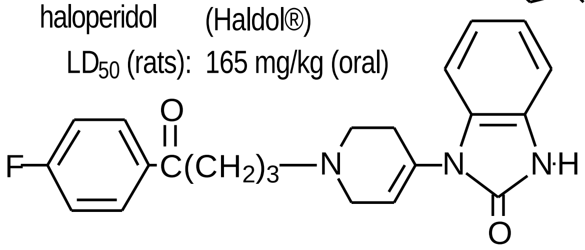
The **butyrophenone** and related diphenylbutylpiperidine antipsychotics include **haloperidol** and **droperidol**. Haloperidol is used not only for mania, dementia and other psychotic conditions but can also be prescribed for Tourette's syndrome, a condition found in children and adults which is characterized by facial twitches, tics and uncontrolled shoulder and arm movements. As the the child gets older he/she may grunt, snort or shout obscenities without control.

Droperidol produces a state of decreased anxiety and attention to surroundings. Combined with the opiod fentanyl, it can be used to induce an analgesic state.



haloperidol (Haldol®)

LD_{50} (rats): 165 mg/kg (oral)



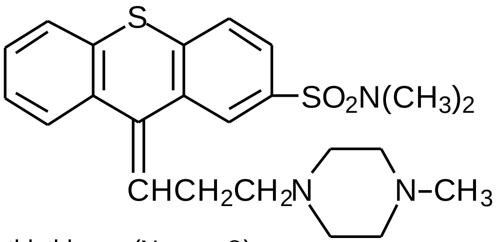
droperidol(Inapsine®)

LD_{50}(mice): 125 mg/kg (s.c.)

43 mg/kg (i.v.)

Lithium in the form of lithium carbonate and citrate (Eskalith®, Lithane®, Lithobid®, Lithonate®, Lithotabs®) is a very effective treatment for manic depression. It has been used since the 1949 discovery of its efficacy by J.F.J. Cade in Australia. Lithium was not fully accepted in the United States until its 1969 FDA approval. Even now its use is limited because of the difficulties in diagnosing and treating manic depression. A problem arises in that the lithium only seems to be effective if treatment begins with the manic state. Then it takes about 3 weeks for the full benefits to be observed, that is, a decrease in the intensity of the swings from extreme excitement to utter despair. Because of the time lag, an antipsychotic drug may be given for a limited period of time at the beginning of lithium dosage. Lithium can cause serious side effects to the kidneys. Nausea, vomiting, diarrhea, trembling, a metallic taste, and dulled responses are common side effects. Obviously, the blood must be monitored on a regular basis to avoid serious side effects. The normal dose is 900 to 2100 mg per day and the duration of action is 18 to 36 hours. The patient should also be careful of sodium intake and increase their water consumption.

Thioxanthenes are related to the phenothiazines and act at DA receptors. Examples of this structural class are chlorprothixene and thiothixene.



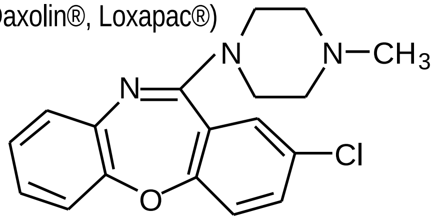
thiothixene (Navane®)

a thioxanthene analog of the phenothiazines

The cis isomer has greater pharmacological activity than the trans and is the one which is used therapeutically.

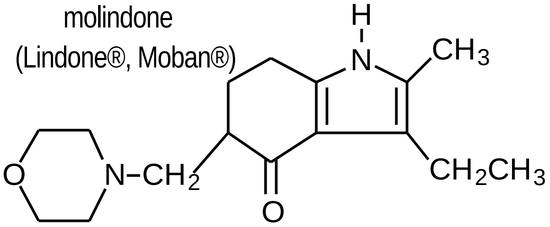
LD_{50} (cis isomer) (mice): 100 mg/kg (i.p.)

loxapine (Loxitane C®, Daxolin®, Loxapac®)



LD_{50}(mice): 65 mg/kg (oral)

molindone (Lindone®, Moban®)

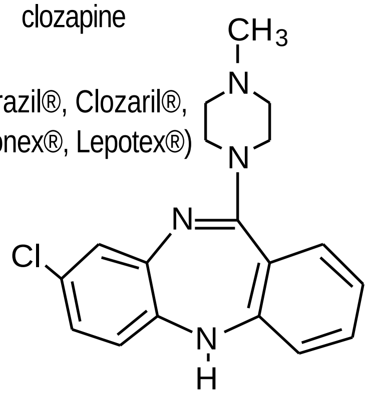


LD_{50}(rats): 261 mg/kg (oral)

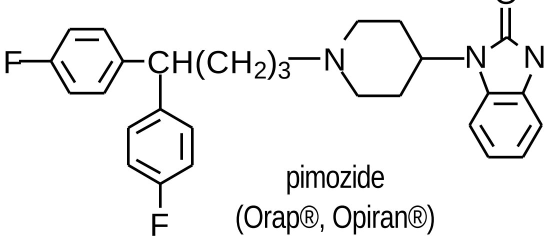
The **dibenzoxapine** loxapine and the **indolone** molindone are also used as antipsychotics.

Clozapine is a benzodiazepine which is considered, along with the diphenylbutylpiperidone pimozide, to be atypical antipsychotics. Introduced in 1951 clozapine was not a drug of choice due to a side effect of agranulocytosis. It became newsworthy in 1990 when it was marketed for the treatment of schizophrenia by Sandoz Pharmaceuticals in conjunction with Roche labs. The treatment became a package deal which included not only the drug but also mandatory blood testing on a regular basis. The cost to the patient - $9000 annually. The effectiveness of clozapine in the treatment of schizophrenia was considered very good especially in light of the fact that the extrapyramidal side effects, compared to other antipsychotics, was minimal. In Europe the joint marketing has cost quite a bit less. Needless to say, the very high charge for the package brought a good deal of notoreity to the drug.

clozapine (Clorazil®, Clozaril®, Leponex®, Lepotex®)



LD_{50}(mice): 61 mg/kg (i.v.)
199 mg/kg (oral)
(rats): 58 mg/kg (i.v.)
260 mg/kg (oral)



pimozide (Orap®, Opiran®)

Pimozide is a potent, long-acting agent also used for Tourette's syndrome.

c. Analgesics

Analgesia means pain relief. Pain is an extremely subjective experience. The biochemical causes of pain are far from understood. The psychological aspect to pain such as its exacerbation by fear or anxiety are totally mysterious. We do know that certain substances such as prostaglandins are produced at the site of an infection or injury and can cause inflammation, swelling and then pain. The pain results from the stimulation of neurons by specific neurotransmitters like Substance P, a polypeptide. Analgesic drugs act by inhibiting the production of prostaglandins or by blocking the actions of pain-neurotransmitters. The former activity is found with non-narcotic analgesics such as aspirin, acetaminophen, and the NSAIDs (nonsteroidal antiinflammatory drugs). The non-narcotic analgesics will be covered in the next part of the course in the section on the immune system.

The narcotic analgesics are also called the opioids because they are related structurally to the natural products of the opium poppy. The medical properties of opium have been known for thousands of years. It has been used by shamans and medicine men and women for pain, sleep, coughing, and diarrhea. The key chemical compound among the 20 or so isolated from the resin of the unripe opium poppy native to countries of the Near East, ***Papaver somniferum*** and ***Papaver album***, is morphine. It was first purified by a German pharmacist, Friedrich Wilhelm Adam Serturner, in 1806 and named morphine because of its sedative effects (Morpheus was the Greek god of sleep).

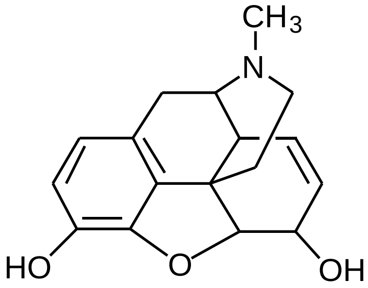
Opium preparations have taken many forms during history. Laudanum was any of a number of tinctures or mixtures of opium with other materials. In the nineteenth century tincture of opium was given the acronym GOM (God's Own Medicine). Opium was smoked and eaten recreationally and to boost the output of natives at hard labor thoughout the Near and Far East for hundreds of years.

The British brought the opium habit back to England and then launched military excursions in order to keep their opium trade from India to China alive (the Opium Wars, 1839-1842, 1856-1860). A group of English literary giants were known as the "opium eaters" - Coleridge, Byron, Shelley, Browning, Dickens, and de Quincey. The invention of the hyperdermic syringe in 1856 introduced another method of administration with more punch - "mainlining" and the benefactors of that mode of introduction in the U.S. were the soldiers injured during the Civil War.

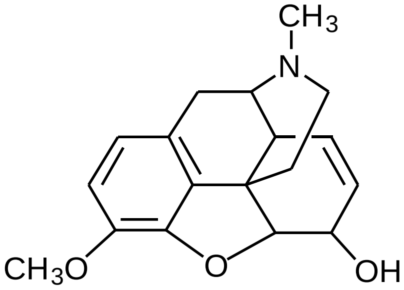
We are all acutely aware of the abuse potential of morphine and its derivatives. Although morphine is still a necessary agent in the physician's drug formulary, heroin is a scourge as are some of the so called "designer drug" opiates which are even more potent.

Narcotic analgesics have an extremely high possibility for addiction, tolerance and physical dependence. Withdrawal reactions are very unpleasant to say the least (stomach cramps, nausea, vomiting, diarrhea, and treamors) but are usually not life-threatening. Chronic abuse leaves the victims with constipation, lethargy, decreased respiration and pinpoint pupils (no tolerance is built up to the last). There is a cross-tolerance among the members of this class of drugs indicating a common mode of action.

The mechanism of action of the opiates was elucidated within the past twenty years and is still the subject of intense investigation. It was proposed that since exogenous compounds in small doses can relieve pain, there must be endogenous neurotransmitters and receptors responsible for the alleviation of pain. These agents were dubbed endorphins (endogenous morphines) and enkephalins ("in the head") and have been identified as polypeptides derived from larger precursor proteins. Many types of opiate receptors have been studied and they have differing affinities for synthetic and natural opiates are located throughout the body.

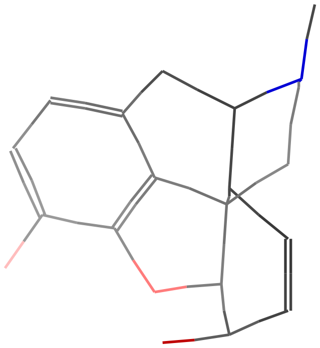


morphine
LD_{50}(mice): 500 mg/kg (s.c.)

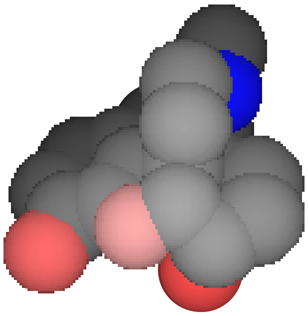


codeine
LD_{50}(HCl salt) (mice): 300 mg/kg (s.c.)
(phosphate)(rabbits): 100 mg/kg (oral)

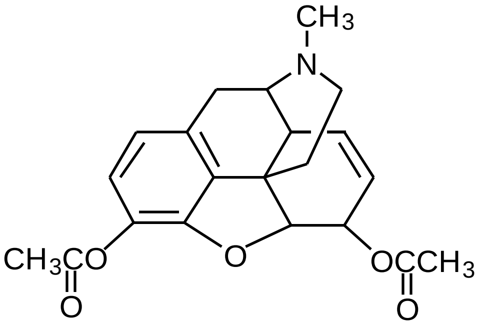
Morphine and **codeine** are both isolated from opium. Codeine (Schedule II), less potent that morphine, is biotransformed into morphine in the body and then further metabolized.



stick model of morphine



space-filling model of morphine

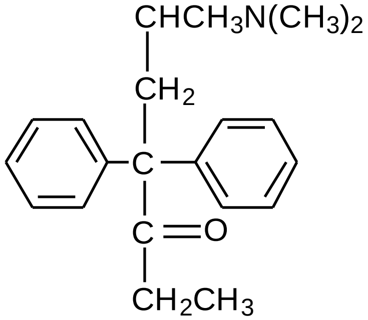


heroin (diacetylmorphine)
The fatal dose in mice is 262 mg/kg given subcutaneously.

Heroin (Schedule I) is the acetylated form of morphine. The derivatization makes it more lipophilic and heroin therefore has about three times the potency of its morphine parent. Street heroin is rarely more than 10% heroin, the rest being some type of filler. The summer of 1989 saw what the L.A. Times reported as an "explosion" of Southeast Asian heroin with average purities of 48% to 51%, much higher than is expected by the user. The purer heroin makes it easier to smoke, a practice used by crack addicts to modulate the cocaine high. Recall that a "speedball" is a mixture of cocaine and heroin, the cocaine a powerful stimulant and the heroin a depressant, taken in combination to offset the extremes of the coke. Both are euphorics and so complement each other in that regard.

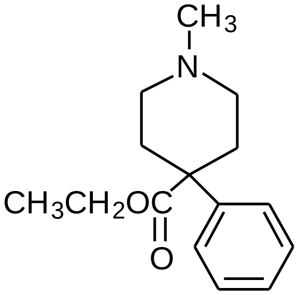
There has been a lot of controversy concerning the decriminalization of drugs, including heroin, as a method of controlling the use of addicting drugs. Great Britain has done so, and in a noble experiment, found that addicts usually were not rehabilitated and their habits became worse rather than better.

Methadone (Schedule II) is an opiate used to ease the withdrawal from heroin addiction. It is itself addicting but does not produce the euphoria of heroin. In addition it can be given orally, thereby discouraging the romance which addicts have with a syringe. The oral administration extends its duration of action and lowers the potency of the drug. The symptoms of withdrawal from methadone commence more slowly and end in a shorter overall period of time than with heroin. There is much contradictory information about the efficacy of methadone treatment programs. The addicts do not like having to report on a regular basis for the oral doses. Many do not take the drug, but instead save it and sell it to other addicts. It is difficult to ascertain whether such programs have had much success overall. It is interesting to note that the rate of recidivism for heroin addicts and alcoholics is about the same.



methadone
(Dolophine®, Fenadone®, Phenadone®)
LD_{50}(rats): 95 mg/kg (oral)

An interesting mixture was concocted in Great Britain's Brompton Hospital to keep cancer patients pain-free and in a euphoric state. Named "Brompton's Cocktail" it consists of: morphine or methadone plus cocaine or amphetamine plus syrup or honey plus 90-98% ethanol or gin plus chloroform and water.

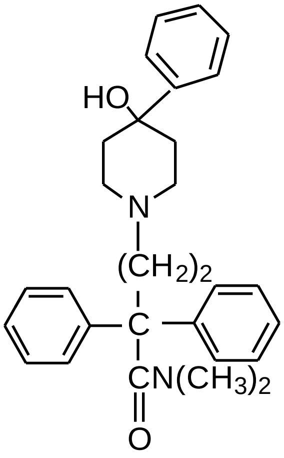


meperidine

(Demerol®, Spasmedal®)

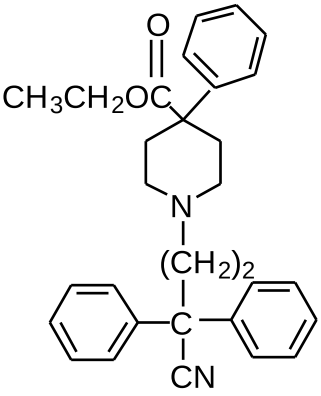
LD_{50}(rats): 170 mg/kg (oral)

Meperidine (Schedule II) is a synthetic analgesic with opiate activity. It has 10-20% of the potency of morphine with all of the addictive side effects. It has a rapid onset of action and duration which makes its useful for relieving the pain associated with labor or as a preanesthetic before surgery. Its biotransformation produces normeperidine which has been associated with seizures. It was a botched clandestine attempt to synthesize a meperidine modification which produced the toxic drug that induced parkinson-type destruction of DA neurons in its users.

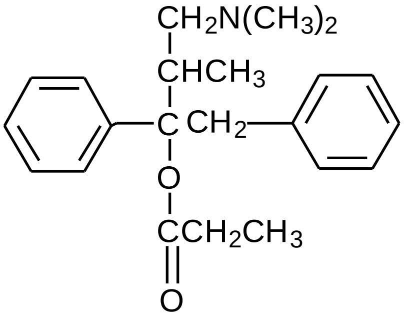


loperamide

Opiates produce constipation by affecting receptors in the intestines. Opium extracts were used in this capacity to treat diarrhea. Today there are other related compounds on the market which accomplish the peripheral task without affecting the CNS because of their poor absorption from the GI tract when taken orally. Imodium A-D®, an OTC, contains loperamide. It is also available as a generic OTC. The prescription mixture of diphenoxylate and atropine is called Lomotil®.



diphenoxylate



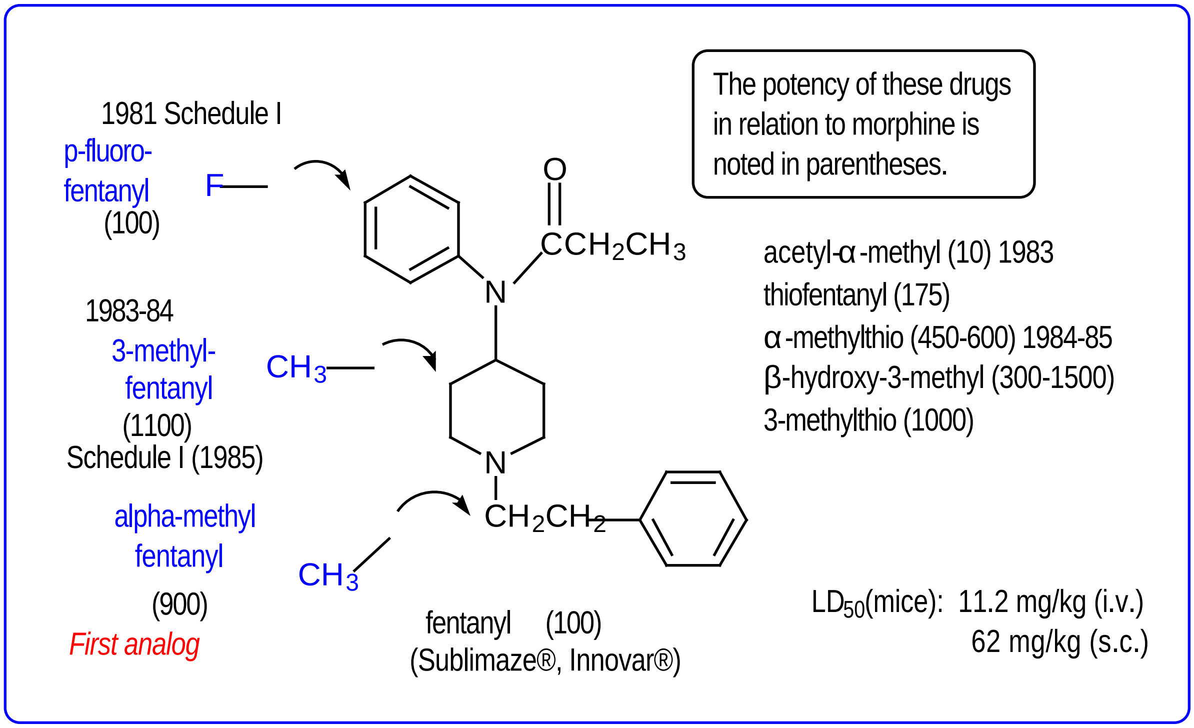
propoxyphene

(Darvon®, Femadol®)

LD_{50}(rats): 990 mg/kg (oral)

Propoxyphene (Schedule II) is another opiate prescribed for pain which is much weaker than those mentioned above. Notice the structural similarities to methadone. Its effects last longer than many other drugs in this class which means it can be taken less frequently and the potential of abuse will be lessened. It can also be formulated with aspirin or acetaminophen in order to have a summation effect.

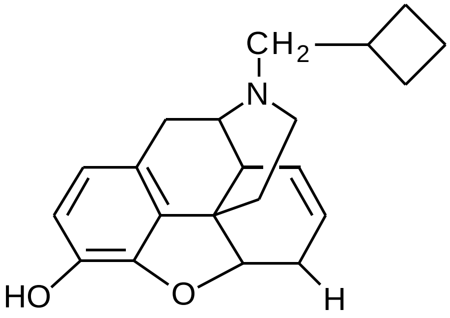
Fentanyl and its derivatives are referred to as "designer drugs" because of the extensive chemical manipulation which has produced much more potent derivatives. Fentanyl itself is 100 times stronger than morphine and its alpha-methyl analog is 900 times more potent. It was difficult to stop the illegal manufacture of these drugs because the existing drug legislation at the time (1983) allowed legitimate manipulation of chemical structures for pharmacological improvements. It took the 1984 National Narcotic Act and state intervention (1985 in California) to reclassify such nonauthorized products as illegal.



Research still continues into opiate analogs in order to produce pain relievers of high potency and low abuse potential.

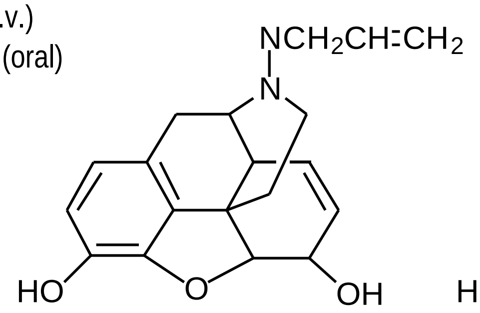
The control of pain has not received the attention which is warranted by the number of sufferers. Surely pain is not an "orphan" disease. Recently there has been a growing concern in the medical community that those suffering from pain because of surgery or terminal cancer, for example, were not receiving the analgesic relief they needed to recover more effectively or to die in peace. This was due to all parties involved: the doctor, who is not fully aware of the extent of the patient's pain and would not prescribed adequate medication; the nurse, who would not administer the drugs prescribed because of busy schedules and inattentiveness to the pain of the patient; and the patient him/her self, who though it could be addicting or that it is noble to suffer. It is interesting to note that the incidence of addiction to opiates by those who have used them under prescription is inconsequentially low. There is ample evidence of the need for an "addictive personality" type in order to produce an addict.

Another ethical issue which relates to these compounds is the "Right to Die" concern. Should doctors or nurses be able to assist terminally ill patients in their death either actively or passively?

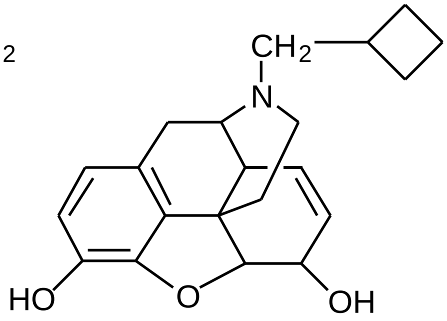


butorphanol (Stadol®)
LD_{50}(mice): 40-57 mg/kg (i.v.)
395-527 mg/kg (oral)
(rats): 17-20 mg/kg (i.v.)
570-756 mg/kg (oral)

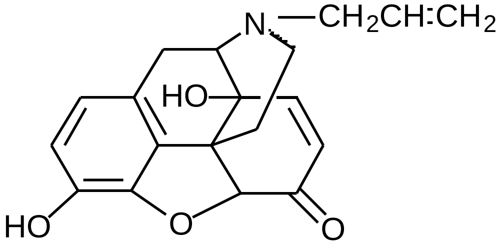
The next four drugs are what are referred to as **"mixed agonists-antagonists"**. Although all can be abused, the potential is lower for the normal person because of the lowered potency. However, the addict will experience withdrawal symptoms either from the drug itself or if the drug is taken with heroin or morphine because of the competition for opiate receptors.



nalorphine (Lethindrone®)
removed from use in 1983

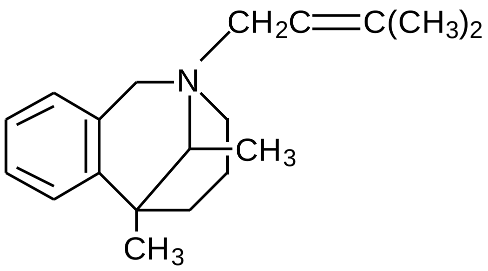


nalbuphine (Nubain®)

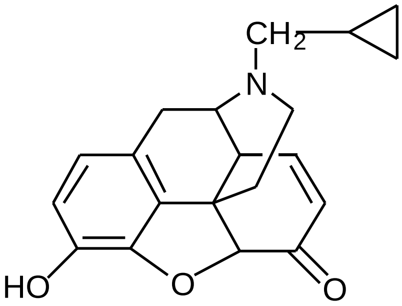


naloxone (Narcon®)

Naloxone is an opiate antagonist which is used as an antidote to opiate overdoses. It has also been used in withdrawal programs, for babies born to addicted mothers, and in the study of the body's natural opiates, the endorphins and enkephalins.



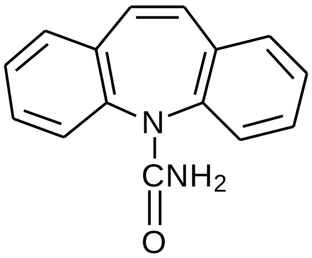
pentazocine (Talwin®)
LD_{50}(rats): 175 mg/kg (s.c.)



naltrexone (Trexan®)
LD_{50}(mice): 586 mg/kg (s.c.)

Naltrexone is also used in the treatment of dependent individuals.

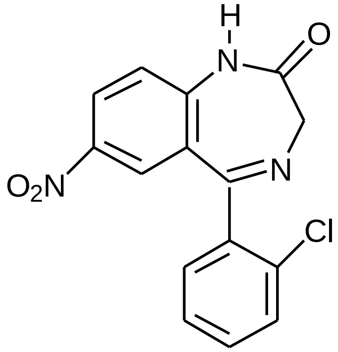
d. Anticonvulsives



carbamazepine
(Stazepine®, Tegretol®)
LD_{50}(mice): 3750 mg/kg (oral)

Epilepsy involves the synchronous firing of nerve cells resulting in sudden, brief (or longer) attacks of altered consciousness, motor activity, sensory phenomena, or inappropriate behavior. There are several types of epilepsy and each has its own particular regime of drug treatment. Grand mal (tonic/clonic) epilepsy is the most severe form in which the victim will experience some warning sensation such as flashing lights or a sound and then lose consciousness. They will have convulsions which are usually short in duration (few minutes) but can be extended (up to one hour - called status epilepticus). The drugs phenobarbital, phenytoin, primidone and cabamazepine are used to control such grand mal episodes and an injection of diazepam can be used to treat status epilepticus.

Petit mal is the most common form of epilepsy found in children. Convulsions are not common, rather the child will seem to "blank out". Succinimides, such as ethosuximide or valproic acid or clonazepam, can be used to prevent such occurrences.

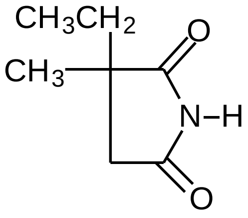


clonazepam
(Clonopin®)
LD_{50}(mice): >4000 mg/kg (oral)

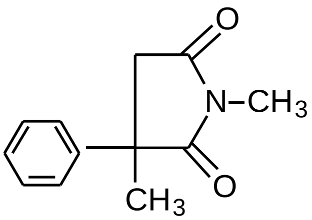
Among the various other types of epilepsy are partial seizures which involve some involuntary muscle spasm or sensual disturbance without loss of consciousness. Phenobarbital, diazepam, phenytoin and carbamazepine are the most commonly used medications for this condition. As evidence of the toxicity and potency of these drugs, carbamazepine has been found to cause spina bifuda in babies born to women who have used it during pregnancy.

Phenobarbital was covered with the barbiturates and diazepam is a benzodiazepine tranquilizer.

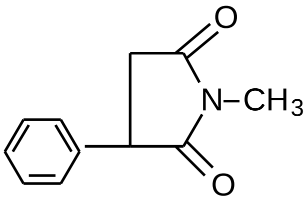
Theories abound concerning the biochemical basis for epilepsy but no definitive evidence lends itself to any one of them. Grand mal seems to have associated with it high concentrations of the enzyme beta glucuronidase in the blood. This enzyme degrades glycosaminoglycans in nerve cell receptors. These complex carbohydrates seem to be important in the electrical discharging of neurons. Epileptics in general secrete abnormally high levels of polysaccharides in their urine. Someday all of these facts may fit into some coherent picture of the condition.



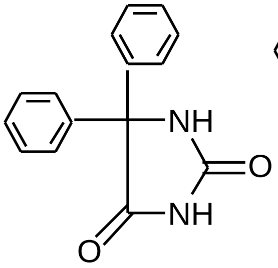
ethosuximide (Epileo Petitmal®, Mesentol®, Zarontin®)
LD_{50}(rats): 1820 mg/kg (oral)



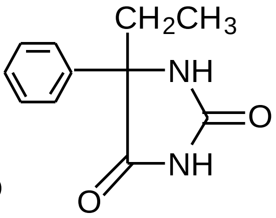
methsuximide (Celontin®,Petinutin®)
LD_{50}(mice): 1550 mg/kg (oral)



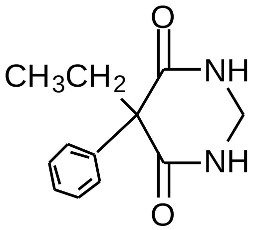
phensuximide (Milontin®)
LD_{50}(mice): 960 mg/kg (oral)



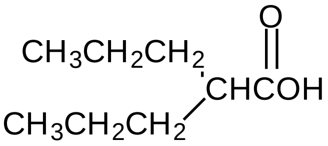
phenytoin (Dilantin®)
LD_{50}(mice): 92 mg/kg (i.v.)
Listed as a carcinogen by the National Toxicology Program in 1981



mephenytoin (Mesantoin®)
LD_{100}(rats): 270 mg/kg (i.p.)



primidone (Myidone®, Mysoline®)
(partially converted to phenobarbital in the body)

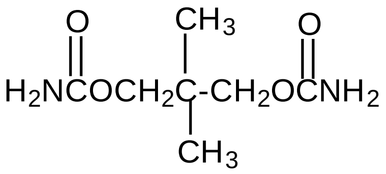


valproic acid
(Depakene®)

e. Ethanol is the most potent OTC depressant which is available. It is a known teratogen (fetal alcohol syndrome) and cocarcinogen. It's abuse results in billions of dollars lost in medical expenses, productivity and untold human heartache. Chronic alcoholism upsets the balance of metabolism producing cirrhosis of the liver, malnutrition, cardiovascular disease, and mental illness (Korsakoff's syndrome). It induces the liver microsomal enzymes and can interfere with the normal biotransformation of dietary materials and other drugs. Oddly enough, ethanol can be used as an antidote to methanol and ethylene glycol (antifreeze) poisoning because it will competitively inhibit their biotransformation.

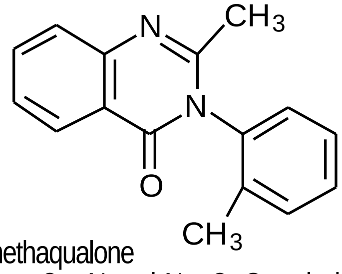
f. Other depressants

Other types of compounds have made the scene as CNS depressants. The severe side effects accompanying their use makes them undesirable as a drug of choice. Several have and still continue to be abused.



meprobamate
(Miltown®, Equinil®)

Meprobamate was developed early in the history of antianxiety medication (1954). Its addictive potential led to experimentation with other types of compounds.

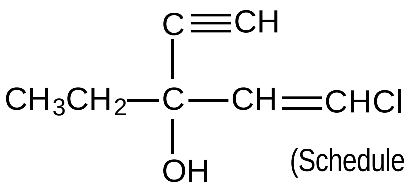


methaqualone
(Sopor®m Normi-Nox®, Quaaludes®)

(Schedule I)

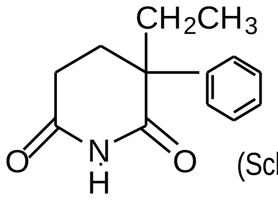
Methaqualone or Quaaludes® was usually prescribed for its hypnotic properties and long duration of action. It was compounded with codeine in order to enchance the analgesic effect of the latter. Its propensity for a prolonged effect made it an excellent candidate for abuse. It was also touted as an aphrodisiac because its hypnotic effects were (and still are) believed to promote unreserved person interactions. A chronic user could be subject to grand mal seizures if the drug was stopped suddenly.

Ethchlorvynol is a Schedule IV drug used for the short term management of insomnia. It produces the same type of tolerance and addiction seen with other depressants.



(Schedule IV)

ethchlorvynol
(Placidyl®, Normoson®)
LD_{50}(mice): 290 mg/kg (oral)



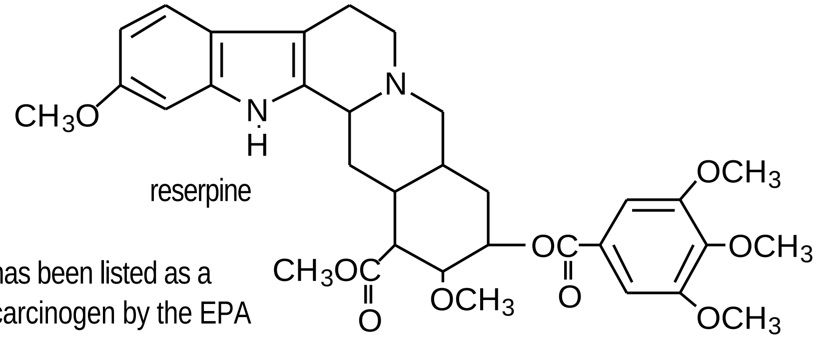
(Schedule III)

glutethimide (Doriden®)

Glutethimide has strong sedative activity and is an inducer of the liver microsomal enzymes. As with all the other depressants it is abused. Especially noted were incidences in Southern California in 1982 wherein abusers would take handfuls of Empirin IV, a mixture of glutethimide and codeine. The practice was called "loading". Since bogus pills were on the market, the potency of the "loads" was frequently very low leading to larger doses attempted. It is fairly obvious deaths occurred when a real supply of Empirin IV was available.

Reserpine is a raulwolfia alkaloid isolated from the roots of the plant ***Rauwolfia Serpentina***. It is used for the treatment of mild essential hypertension or psychosis.

Reserpine has several putative modes of action including the blocking of NE reuptake, inhibition of monoamine oxidases, and depletion of NE and 5-HT stores. The drug comes pure or as the whole root which is biologically standardized. Its lipophilicity allows it to be stored in body fat and it can easily pass the blood-brain barrier as well as the placental membrane. The side effects of reserpine treatment can be severe: hypotension, impaired libido, increased GI motility, respiratory sensitivity, parkinson syndrome, and depression. The depression can occur even after reserpine use has terminated and may lead to suicide. Needless to say this is not a drug of choice. The phenothiazines are more approriate as a first attempt at drug treatment for psychosis.



reserpine

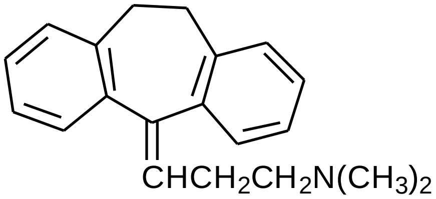
has been listed as a carcinogen by the EPA

5. Antidepressants

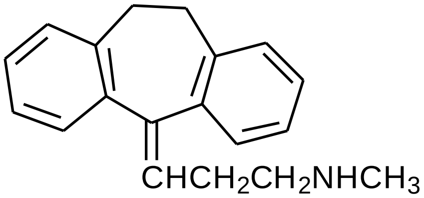
Depression is an incapacitating condition wherein a person is in the midst of despair, lethargy, loss of interest, sex drive, and perhaps even the will to live. It is theorized that a lack of NE and DA is the cause of such a mental state and that either stimulating these systems or preventing their destruction or reuptake reinstates the balance in the CNS.

The **tricyclic antidepressants** are the most widely used class of drugs used for clinical depression and are usually the first type of medication prescribed. All of the effects are not uniform throughout this structural class and the actual prescription may involve an evaluation of some of the symptoms exhibited by the patient. Because the chemical

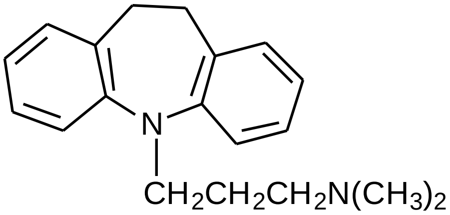
imbalance can be severe it takes quite a while, 10-14 days, for the antidepressant effects to begin to be experienced. A tricyclic such as imipramine, for example, might be given to a depressive who is very lethargic because it will act mainly as a stimulant. Amitriptyline has sedative properties and would bring on sleep early in treatment to an insomniac. Most of these drugs have anticholinergic side effects such as dry mouth and problems with vision which appear early in the treatment. An overdose of these potent substances can produce seizures, coma and heart arrhythmias which could result in death. Acute poisoning is common.



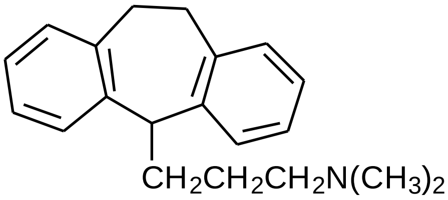
amitriptyline (Elavil®, Endep®)
Limitrol® contains Librium and Elavil



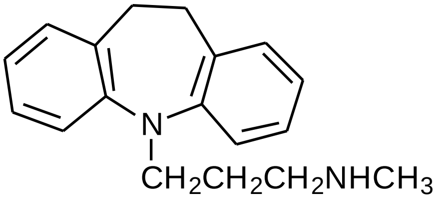
nortriptyline (Aventyl®, Pamelor®)



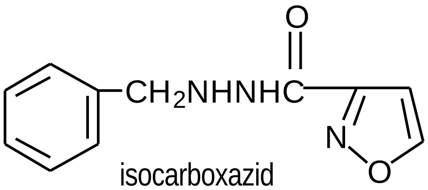
imipramine (Presamine®, Tofranil®)



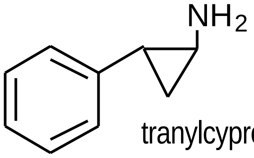
protryptyline (Vivactyl®)



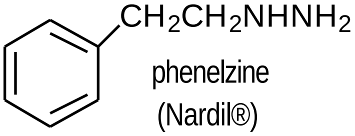
desipramine (Norpramin®)



isocarboxazid (Marplan®)



tranylcypromine

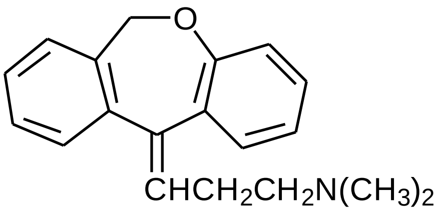


phenelzine (Nardil®)

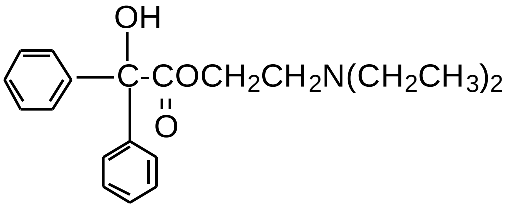
Monoamine oxidase inhibitors (MAOs) are not the drugs of first choice in treating depression. They are prescribed for those who either do not respond to tricyclics or cannot take tricyclics for some reason, for example, those with phobias respond better to MAOs. Because these drugs are enzyme inhibitors of neurotransmitter systems which are key to both the CNS and the PNS, the side effects are not inconsequential. Overdoses are extremely dangerous. MAOs have overdose effects similar to those of tricyclics. The diet of a person taking these drugs must be free of tyramine (red wine, cheddar cheese, herring, etc.) because of the inhibition of tyramine breakdown which can produce severe hypertension and possibly cerebral hemmorhage.

Neither tricyclics nor MAOs should be discontinued abruptly. Withdrawal symptoms such as insomnia and anxiety can occur even under medical supervision.

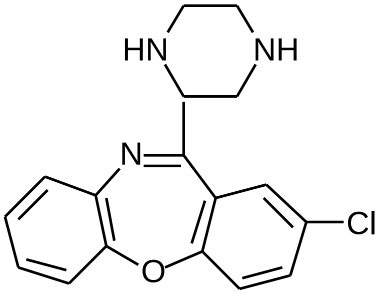
Mild Antidepressants - Many people, young and old, suffer from depression: not the deep, dark abyss of the unipolar depressive or bipolar manic depressive; not the temporary setback as we have all experienced with the loss of a loved one, job or grade; but rather a chronic difficulty in concentrating or enjoying life due to a minor chemical imbalance. The imbalance might be due to a hormonal deficiency, stress, or medication. This type of depression affects more people than you might think. The drugs already mentioned are too potent for such patients. A number of milder, NE/DA-system stimulating drugs have been developed to bring a finer quality of life to the sufferers. It is of interest to note that none of these drugs produce euphoria or a "high" as do amphetamines. They have been used for years without producing tolerance or addiction. The only problem that exists is their overprescription by physicians avoiding more personal interactions with their patients in order to help find the factors in a patient's life which might be worsening the situation.



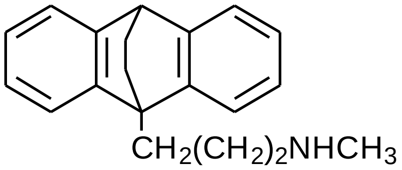
doxepin (Sinequan®, Adepin®)



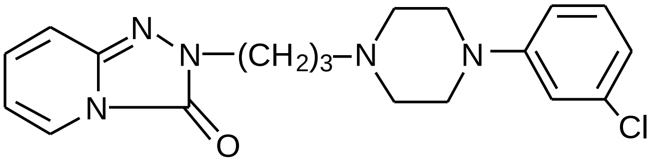
benactyzine (Finalin®, Spatomac®)



amoxipen (Asendin®)

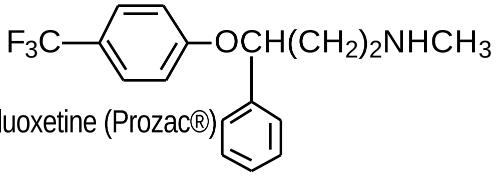


maprotiline (Ludiomil®)



trazodone (Desyrel®)

Prozac® has been the center of controversy over the past few years. Hailed by psychiatrists and psychologists as a valuable tool in the treatment of chronic depression, the press has focused in on its not too infrequent side effects including suicidal depression. Philosophical questions also arise when considering the alleviation of depression viewed as a “natural human condition”. Those on Prozac undergo subtle yet significant changes in motivation and outlook which some believe is abnormal in the scheme of things.



fluoxetine (Prozac®)

This completes our section on the central nervous system and Part Two of the course. By far, more prescriptions are written for drugs affecting the nervous system than any other organ or condition. We also know that the topic of euphoric states either natural or drug-induced stimulates our innate curiosity. I hope that some of your questions have been answered by this section and that you may be motivated to read further on some of these topics.

PART THREE

CHEMOTHERAPY FOR VIRAL AND BACTERIAL INFECTIONS

This last section of our course deals with a number of diverse topics which could be said to have a connecting thread of invasions from exogenous species (bacteria, viruses, chemicals) or internal malfunctions (autoimmunity, cancer). We will deal first with how the body defends itself from nonself. In this discussion we will look at viruses including HIV (human immunodeficiency virus - cause of AIDS). After dealing with a brief look at antiviral drugs we will proceed to antibiotics (antibacterials) and then to a consideration of the causes of and treatments for cancer.

XI. The Immune System

A. Natural Protective Agents

1. Physical

a. Skin - The skin is a natural barrier comprised of several layers which are complex in their biochemical makeup. The outer epidermal layer contains dead and keratinized cells while the inner or dermal layer has most of the extracellular collageous material as well as dermatan sulfate. This dermis also houses the skin's blood supply, a lymphatic drainage system, sweat glands, nerve cell endings, fat cells, and hair cells. Should the skin be broached by a cut or abrasion, the body has lost its first and largest (in area) line of defense.

b. Mucous Membranes - We have briefly touched on mucous membranes in the section on drug administration. The cells of these membranes, which line the air passages, lungs, vaginal and anal surfaces, can secrete proteoglycans (mucous) which can trap exogenous materials as well as contain some of the chemical agents mentioned below.

c. Cilia - Again, as mentioned in previous sections, the bronchotracheal tract contains sensitive hair-like projections which respond to particulate matter and trigger reflexes such as sneezing and coughing which are meant to physically expel the intruder.

2. Biochemical

Various proteins have mechanisms of action which can destroy or incapacitate invading materials. Lysozyme, for example, is found in tears and it breaks apart bacterial cell walls, thus neutralizing the agent. Basic

proteins known as histones and protamines also have antibacterial action. Certain glycoproteins in the blood show antiviral activity.

B. The Composition of the Immune System

An immune response is a coordinated effort of cells and tissues which initiates and sustains a defense against chemical and organism not native to the host organism. It is protective against what is referred to as "nonself". Nonself can include bacteria, viruses, parasites, fungii, foreign tissue, and cancer cells.

The lymph is a system of circulating materials which exists alongside the blood supply. As the metabolites and other species in blood are drained into the tissues, the used fluids must pass through and out of the tissues. The exudate goes into the lymph system which eventually drains through the thoracic duct in the upper portion of the chest back into the blood supply. It is important to remember this connectivity of the blood and lymph and its relation to the tissues in order to appreciatie the complexities of the immune system.

1. Humoral System - Circulating portions of the immune system, called the humoral system, include the blood and lymph.

2. Tissues - Tissues involved in the immune system, the cellular system, are the: lymph nodes, located in the neck, underarms and groin areas; spleen, above the kidneys; tonsils and adenoids; and Peyer's patches, found in the area of the small intestine as well as the appendix located near the large intestine. All of these are located at key places in the body so as to interact with the available blood supply.

3. Cells - A host of different cells are also involved in this complicated defense system - B and T lymphocyctes, macrophages, granulocytes, and mast cells, for example.

4. Complement - A network of proteins known as complement also contribute to the process in a manner somewhat analogous to the blood clotting cascade. The difference is that this cascade destroys nonself.

5. Cytokines - polypeptides secreted by monocytes and lymphocytes(including T and B cells, mast cells,...) in response to nonself, tumors, etc.

6. Biomolecules - Other types of molecules and macromolecules are active during the immune response. Histamine and serotonin were encountered in our discussion of the nervous system. They are factors which act to intensify the response to a nonself presence. Heparin, the anticoagulant,

and moelcules known as chemoattractants are also present, as are lipid derivatives known as leukotrienes.

6. Terms - There are specific terms used for the biochemical and cellular components of the immune system. The following are the key ones to remember.

a. **antigen (Ag)** - any protein, carbohydrate, or nucleic acid which can elicit an immune response. There is a minimum size which must be present to trigger the response without assistance.

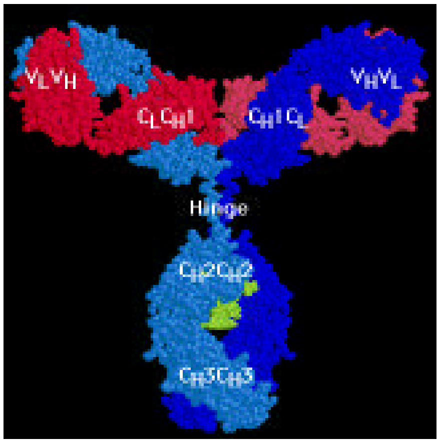
b. **antigenic determinants** - the chemical structures on an antigen which are recognizable to the immune system. There may be one or more antigenic determinants per antigen depending upon the size of the latter.

c. **hapten** - a chemical of low molecular weight which of itself cannot cause an immune response but because of its reactivity can combine with the host system's biomolecules, frequently serum albumin, in order to present an antigen of reasonable size. The classic hapten is 2,4-dinitrophenol which binds to albumin and the complex starts an immune reaction.

d. **antibodies (Ab)** - glycoproteins produced by "B-cells" (part of the circulating system) which have affinity for antigens and can either: a) themselves complex with the antigen, form precipitates, leading to the complex being eliminated from the host system; or b) stimulate a more complex response from the immune system resulting in the destruction and elimination of the antigen.

Antibodies are also called immunoglobulins (Igs) and can be found in specific electrophoretic fractions of the blood plasma. Figure 28 diagrams the structure of an antibody.

Each Ab molecule consists of four protein chains - two light chains and two heavy chains. The chains are covalently linked to each other by disulfide bridges. The four chains come together in the shape of a Y with the ends of the arms of the Y, the amino termini of the four polypeptide chains, acting as binding sites for antigen. The lower portions of the antibody contain sites for carbohydrate (CHO) attachment as well as sites of interaction with other factors in the immune system.



Within both of the light and heavy chains are regions of amino acid sequences (primary structures) which are constant, that is, they are the same for each type of antibody molecule we will consider. There are five types of antibodies and five sets of constant amino acid sequences for the heavy chains. The light chains have a choice of two sets of constant regions. More explicitly the five types of constant heavy regions are called α, γ, δ, ε,and μ. The two types of light

constant regions are κ nd λ. The names of the types of antibodies in which they are found are related to the name of the heavy chain constant regions.

Table 5 lists the types of Abs (IgA, IgG, IgD, IgE, and Ig M), their chain compositions, the macromolecular assemblies they participate in, the serum concentration of each, and their locations in the body as well as their ability to "fix complement", which will be expained shortly.

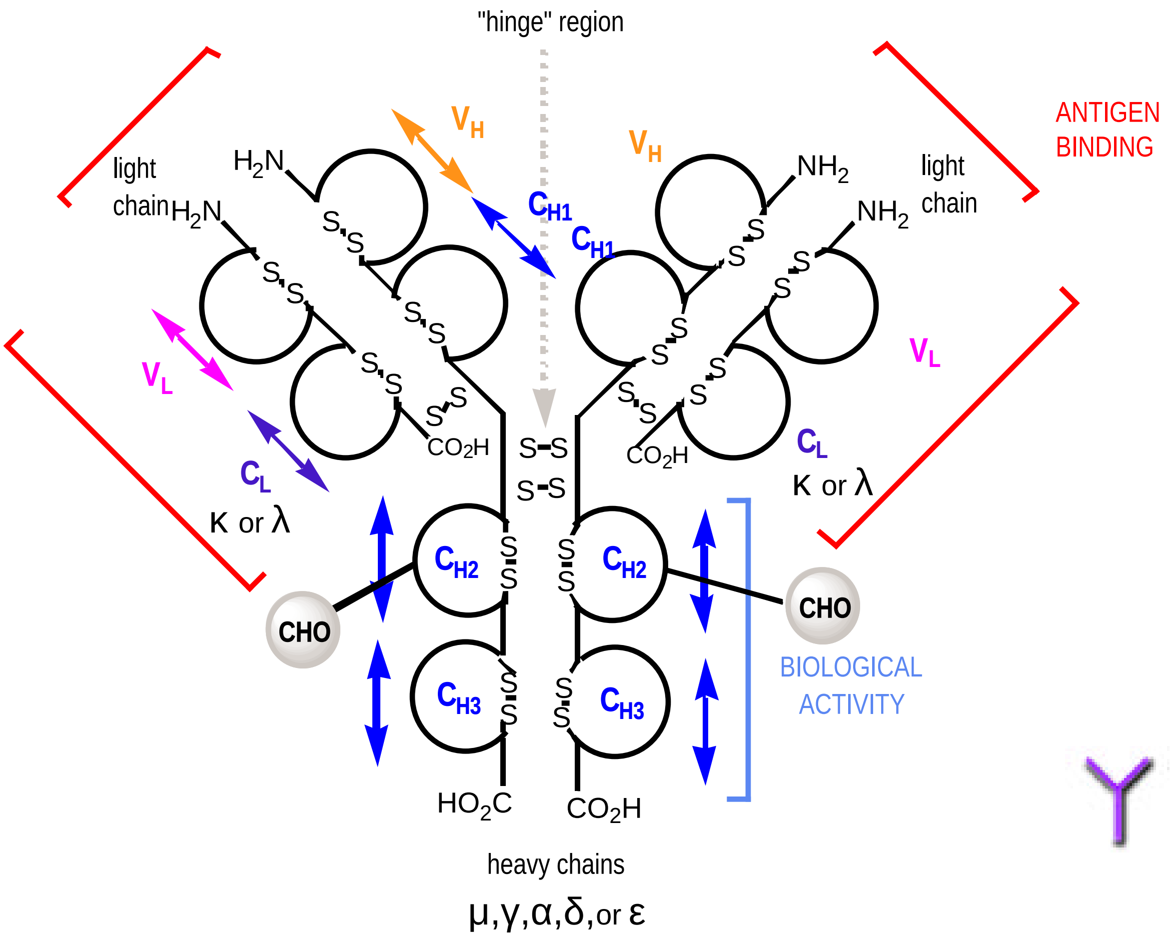
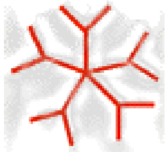
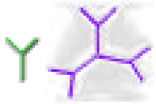


Figure 28
The Structure of Antibodies

The variable regions of the polypeptide chains are just that, variable, that is, they are different and specific for the various antigens they encounter. Genetically the great variety of antibody types are assembled by cutting and splicing messenger RNA segments. This increases the number of possibilities for recombination and leads to a huge number of tailor-made Abs, more than 10^8.

Several Ab types have the ability to "fix complement". This means that the complexes which they form with antigens will attract the blood protein

complex called complement which, once stimulated, will act to destroy the "nonself" entity, usually by the lysis of its cell membranes. Complement is also responsible for the binding of macrophages (the parasites of the immune system). See Figure 30.

Ig Class	[Serum] mg/mL	1^0-3^0 structure	4^0 structure	molecular weight x 10^3	Location Activity
IgG	12	$\kappa_2\gamma_2$, $\lambda_2\gamma_2$	monomeric	150	blood serum; crosses placenta; fixes complement
IgM	1.0	$\kappa_2\mu_2$, $\lambda_2\mu_2$	pentameric 	950	blood serum; fixes complement
IgA	1.8	$\kappa_2\alpha_2$, $\lambda_2\alpha_2$	monomeric to trimeric 	180	external secretions (sweat, tears, mucous, colostrum, saliva)
IgD	0.03	$\kappa_2\delta_2$, $\lambda_2\delta_2$	monomeric	174	blood, gut
IgE	0.0003	$\kappa_2\varepsilon_2$, $\lambda_2\varepsilon_2$	monomeric	200	skin, blood ("allergic" reactions - binds to mast cells which then degranulate releasing various factors)

8. T cells (T lymphocytes)

Essential contributors to the immune response are the T cells. There are several kinds of T cells each with a specific function to perform.

a. **T_H** are so-called "helper" T cells which aid in the transformation of B-cells to plasma cells which bear the antigenic "memory" of the immune system. They are also important to the maturation of other T cells.
b. **T_M** are memory T-cells which retain their own antigenic memory of the first "nonself" invasion.
c. **T_C** are the "killer" or cytotoxic cells

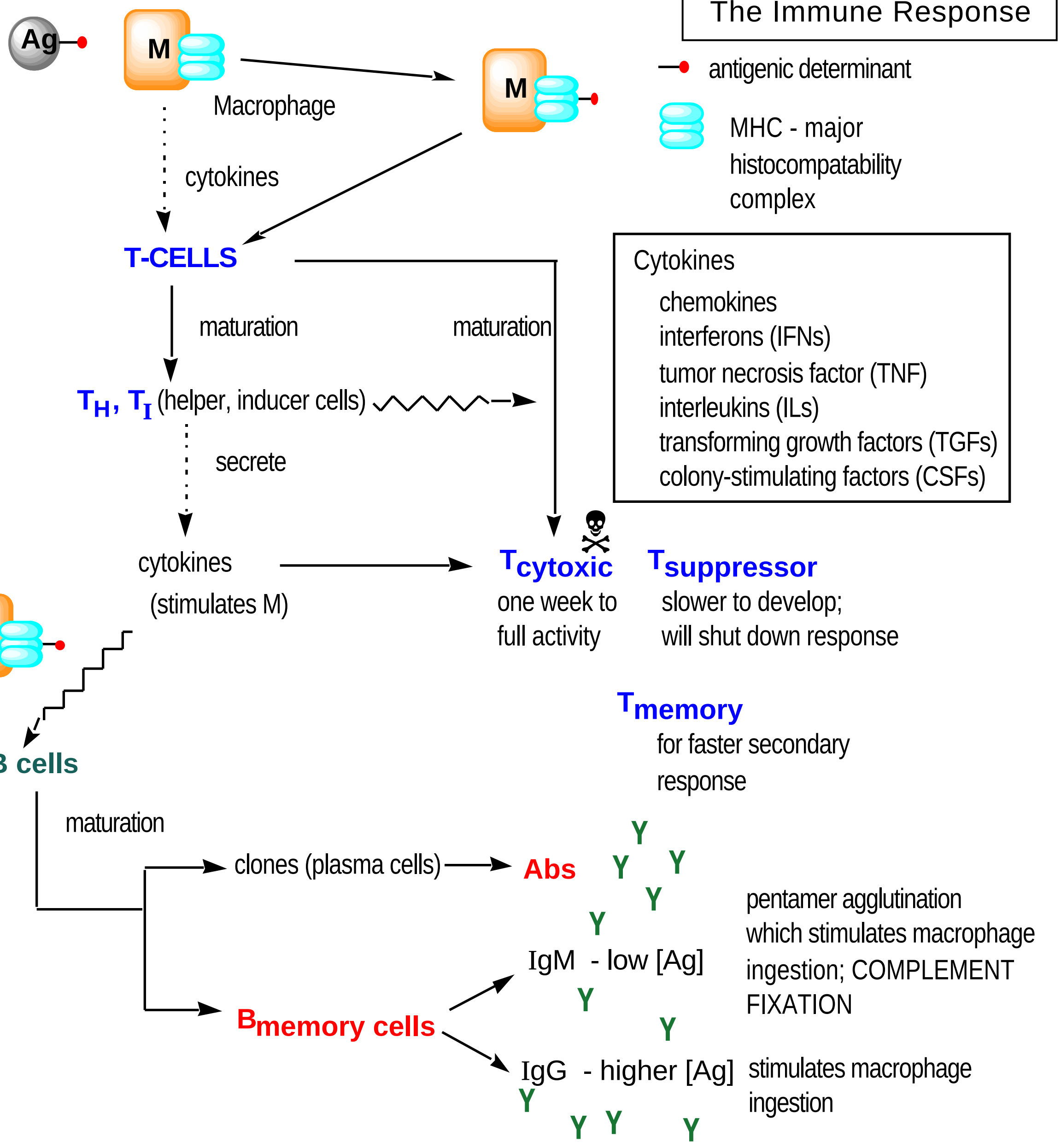
d. T_S stands for the suppressor T cells which suppress an ongoing or developing immune response. They help to "give it a rest".
e. T_I are the inducer cells.
f. T_A cells help in the cytotoxic T cell proliferation.

The T-cells have antibody like polypeptides displayed from and imbedded in their cell membranes.

C. The Immune Response

The components of the immune system contribute to an intricate interplay when an entity foreign to the host organism enters the blood or tissues. Figure 29 outlines the key features of the sequel.

Figure 19
The Immune Response



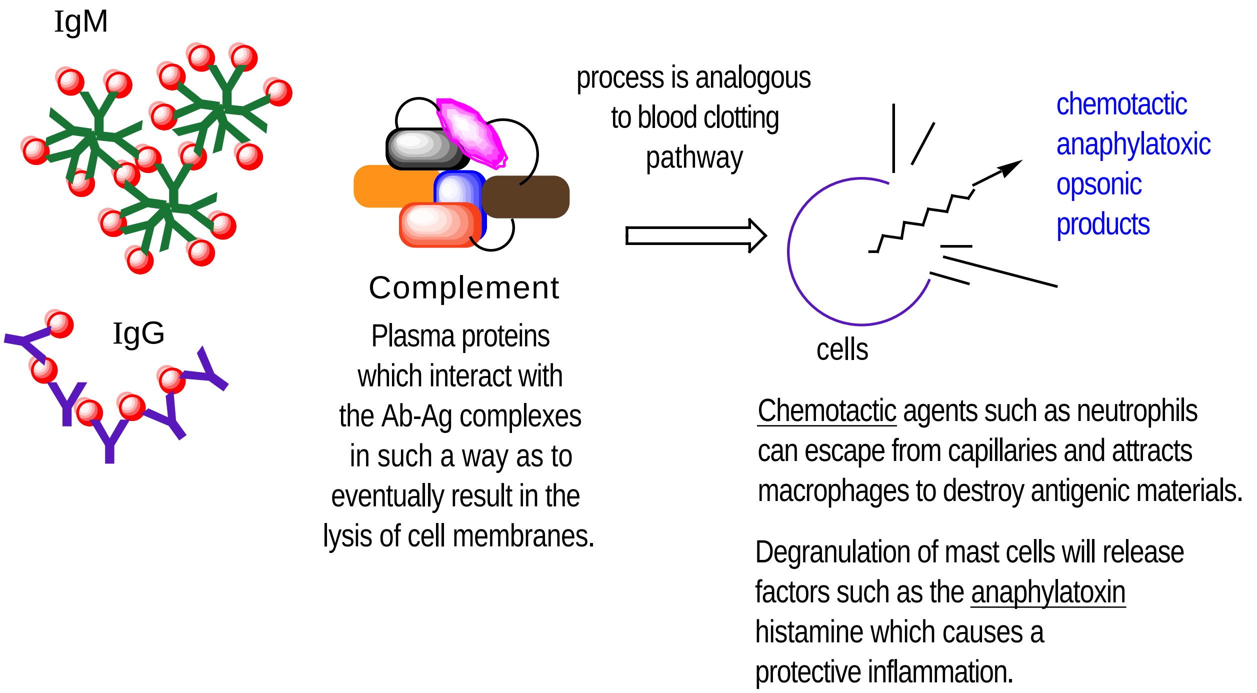
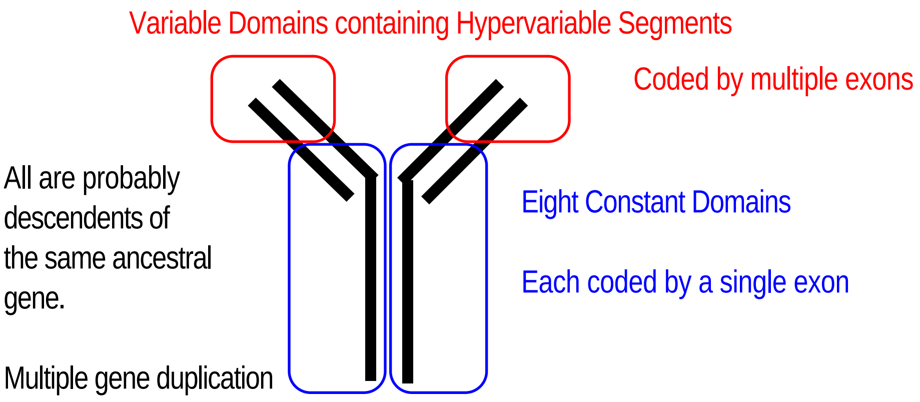


Figure 30
The Actions of Complement

The stimulation of antibody response and production is different for a primary and a secondary antigenic "challenge" or occurrence. The first line of Ab defense is IgM. Its large pentameric structure can bind many antigens and act to remove them from the system. However, the antigenic memory of the T and B cells allows a much faster response the second time around. In fact, the mature B cells will produce IgG specific to the antigen in question and do so in much larger quantities as time goes on. It is important to help the system retain its memory but repeated challenges - "boosters". This concept is important to the concept of immunization by which we are made "immune" to toxic, bacterial and viral attacks. (Figure 31)



IgG is the only antibody type which can pass the placental membrane, a characteristic which allows the newborn infant to have a passive type of immunity. This will be discussed shortly.

The presence of antibodies to a specific agent is evidence of exposure to that antigen. Blood tests can then be devised to test for those antibodies leading to the detection of disease states as well as quantiation of hormonal levels and drug exposures.

For example, ISOLABS, Inc. has a test used to detect IgG in cerebral spinal fluid as an indicator of infection. If no neurological disease exists the concentration of IgG is less than 10% of all CSF proteins. Should multiple sclerosis be present the [IgG] will range from 11-35%. This result plus the results of other confirmatory tests can significantly aid in diagnosis.

Antibodies can be combined with enzymes and color reagents or radioactive antigens to produce quantitative testing for drugs. The ELISA or Enzyme-Linked ImmunoSorbant Assay uses antibodies generated against the Ag to be tested for covalently linked to an enzyme which can catalyze a color change reaction such as the NADH to NAD^+ conversion (λ_{max} at 340 nm). When the Ag-Ab complex is formed the enzyme is activated and the color can be detected.

The RIA or RadioImmunoAssay uses a known quantity of radiolabeled Ag complexed with an equivalent amount of its corresponding Ab. A standard curve is generated with varying amounts of radiolabel Ag. Then the sample to be analyzed, which is nonradioactive, is mixed with the "hot" Ag^*-Ab complex. The "cold" Ag will displace the "hot" Ag to a degree proportional to its concentration in the solution. This is then compared to the standard curve.

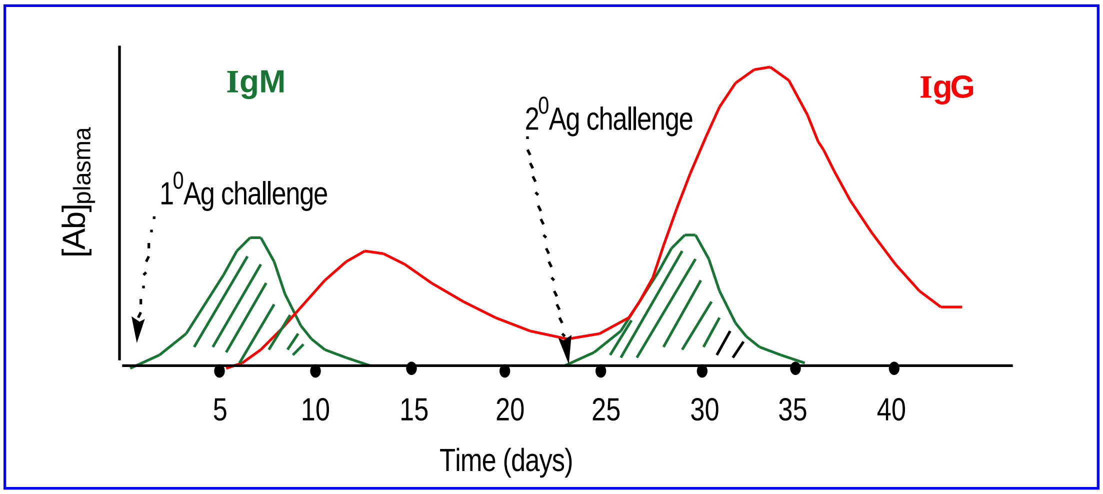


Figure 31
Stimulation of Antibody Production

T and B cells are both made by the bone marrow. They start off as similar "stem" cells and become differentiated as they pass through the tissues of the immune system. Figure 32 illustrates the origin, types, and functions of these cells.

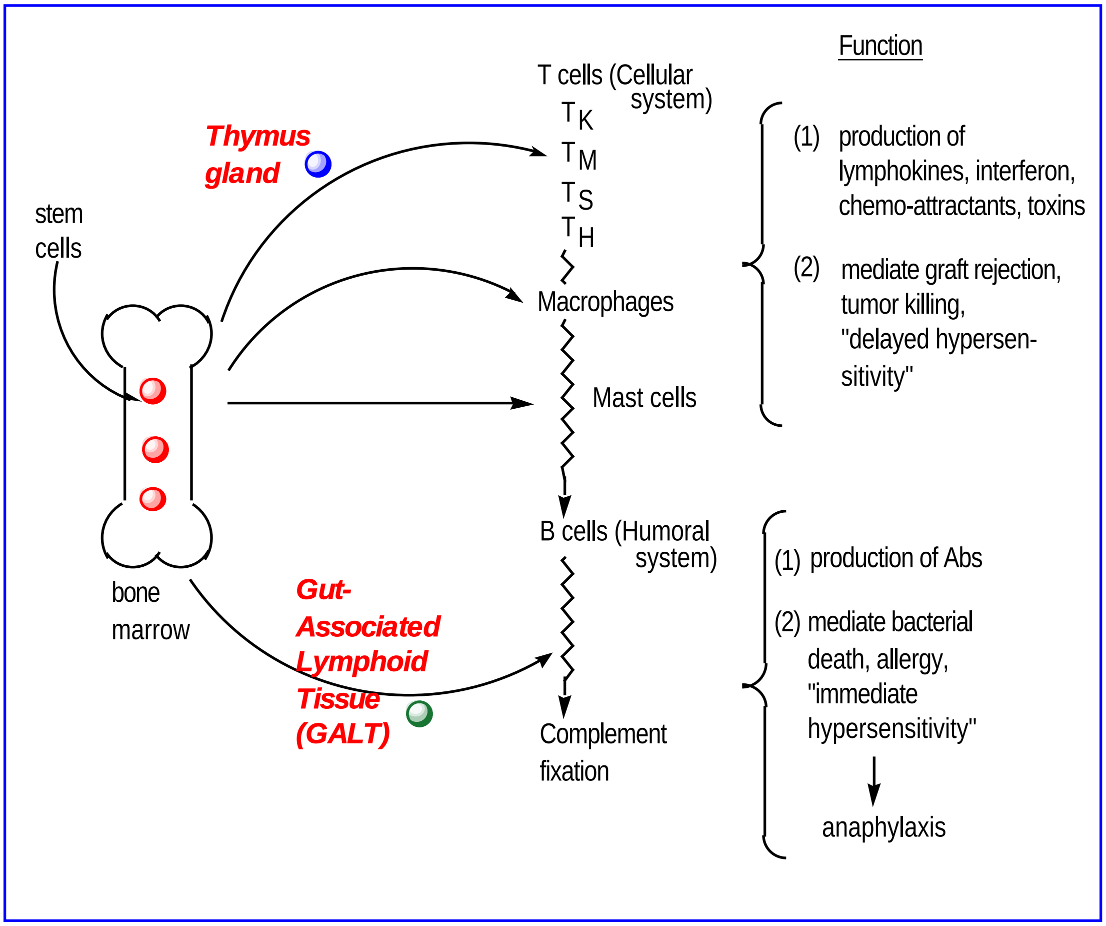


Figure 32
T & B Cell Production

D. Immunity

Immunity means being resistant to attack by nonself. The property of immunity to infection or toxicity can occur naturally or be induced in an organism. Immunity can also be active, that is, involve the T and B cell responses, or it can be passive, that is, antibodies can be infused into the infected organism and so curtail the infection process.

1. Active immunity occurs when the organism is exposed to a toxin or infectious agent (bacterium, virus, or parasite) so that the immune system is stimulated. Most of your parents, grandparents, and instructors contracted measles, mumps and chicken pox when they were children and so are now naturally immune to such agents because their antigenic memory (B and T cells) is primed for a full scale IgG response.

Another way to gain active immunity is to have small amounts of an infectious antigen introduced into the organism so as to challenge the immune system but not cause a full-blown infection or toxic reaction. This is the process of immunization (vaccination if the agent is a virus). In order to minimize an active infection, viruses may be "killed", that is, rendered nonvirulent by heat or chemical treatment, or they may be **attenuated** or treated so that they are not as infectious as usual. The intrusive agent of a bacterium is usually not the bacterium itself but toxins secreted by the organism. Toxins are proteins and therefore antigenic. Heat or chemical treatment of a bacterial protein toxin can eliminate its toxicity but allow its antigenicity to remain. This is called a **toxoid**.

There is a standard series of immunizations which must be undertaken in order to enter the school system, including the CSU, in California.

a. **Standard Immunizations**

D Diptheria (toxoid)
P Pertussis (also known as "whooping cough")*
T Tetanus (toxoid)
H. influenzae, type b
Polio** (Salk, Sabin)
Measles, Mumps, Rubella (German measles)
Hepatitis B

*There has been some controversy concerning the DPT combination shot. Of the three antigens in this mixture the pertussis is the only bacterial agent. It can cause an active infection. The CDC recommends using a safer agent - DtaP - which may contain smaller doses of the diptheria or pertussis toxiods.

**The original polio vaccine was developed by Jonas Salk (for whom the Salk Institute in LaJolla is named). It is a "killed" virus. However, over the years it was found that this did not always impart a complete immunity. The Sabin vaccine contains an attenuated virus. It is interesting to note that the Sabin vaccine can cause an active infection in a rare number of cases. There are more cases of polio caused by the vaccine in the U.S. than are found overall. However the effectiveness of polio immunization is impressive. In 1986 the number of cases of wild type poliomyelitis which occurred in the Western Hemisphere was 930. In 1989 that number went to 130; in September 1989 it was 11 and there have been no new cases of polio in the U.S. due to the environmental agent since 1979. However,

because of the possibility of infants contracting polio from the attentuated Sabin vaccine, the FDA recommends that the Salk vaccine be used for the first two doses.

Meanwhile a new condition has come on the scene which may or may not be related to polio - Chinese paralytic syndrome. This disease involves the motor neurons of the spinal cord which become incapable of generating signals. The name reflects the origin of the syndrome. In Latin American a similar, though not exactly the same, syndrome was reported in 7000 cases between 1987 and 1990.

The current Sabin polio vaccine will give primary immunity within 5-14 days after administration and secondary immunity within 1-3 days after a booster dose.

Other vaccines available are those for influenza(flu), pneumonia (lifetime immunity), rabies, cholera, smallpox, typhus, typhoid, and Rocky Mountain spotted fever. Some vaccines can cause adverse effects especially to small children or the elderly. Flu vaccine has been implicated in the onset of a condition known as Guillain-Barre syndrome, a polyneuritis which can lead to anything from mild pain and weakness in the extremities to paralysis. These symptoms can last from a few weeks to a few months.

2. **Passive immunity** is that imparted by the the natural presence or injection of antibodies to an infectious or toxic agent. Some people are born with inherited immunities due to heritage, diet, metabolism, temperature or adaptive features of existing infectious organisms within the body. A pregnant woman can give passive immunity to her fetus because IgG will pass through the placental membrane into the baby's blood supply. Breast milk supplies IgA and a small amount of IgM.

Injected forms of antibodies which have been generated in another body or animal can be isolated, purified, and administered as standard human immune serum globulin (ISG), and ISG plus preparation, or as an animal antiserum or antitoxin. Some serums which are available are those for rabies, snake and insect bites, botulism, and tetanus. Temporary immunity of up to six months to hepatitis can be imparted by one "gamma globulin" shot. More permanent active immunity is available to health care workers.

The lifetimes of serum antibodies are limited. For example, those generated by humans have a half-life of about 30 days while those from horses or cattle have a half-life of about 7 days. A serious consideration is that human and animal antibodies are different proteins and the human may develop an immune response to the foreign antibody.

E. Autoimmunity

The immune system in each individual develops in such a way that if an antigen from the "self" is encountered by an immature lymphocyte, the lymphocyte is destroyed by a mechanism which we do not yet understand.

Therefore we do not normally develop antibodies and cellular immune responses to our own antigens. However, the immune system in its complexity can go awry. When this happens an organism begins to destroy itself. Much is currently under investigation concerning these "autoimmune diseases".

Examples of some conditions which are known,or are believed to be, to be autoimmune responses include myasthenia gravis (destruction of acetylcholine receptors), rheumatic fever (a streptococcal infection challenges the immune system and then the immune system mistakes heart tissue for another strep infection), Addison's disease (destruction of the adrenal glands), arthritis (an infection of unknown origin starts the immune response but somehow IgG becomes changed, enough so as to start another IgM response - this time to the body's own IgG), pernicious anemia (inability to process vitamin B_{12}), insulin-dependent diabetes mellitus (IDDM or type I diabetes), multiple sclerosis, aspermatogenesis, and photosensitivity.

Conditions which can affect the immune system and cause it to malfunction are Cushing's disease (caused by excess steroid production or treatment - T and B cell lysis), Hodgkin's disease (cancer of the lymph nodes - whole body T cell deficiency), Bruton's disease (B cell deficiency - susceptibility to infection), and, of course, AIDS (Acquired Immune Deficiency Syndrome).

For more on autoimmunity you can read the article in **Science 248** pp. 1335-1393 (June 15, 1990).

F. AIDS (Acquired Immune Deficiency Syndrome)

The cause of AIDS is a retrovirus (RNA containing virus which directs the formation of its own DNA which is then incorporated into the DNA of the host cell) called HIV (Human Immunodeficiency Virus). There are two forms of HIV currently known - HIV 1 and HIV 2. The target of the virus is the T_4 lymphocyte necessary for mounting an immune response. The virus gains access by using the CD4 receptor of the T_4 cell. Since these are retroviruses it takes time for the incorporation of the virally-induced DNA into the host genes. There is also a latent period in which no active infection is evident although the host body will show antibodies to HIV. This latency period can extend from several months to several years. Another event will trigger the viral DNA to proliferate and destroy the host T lymphocytes. As a result, the victim will have a deficient immune system and be susceptible to opportunistic infections, most commonly pneumonia caused by ***Pneumocystis carinii,*** a protozoan, or tuberculosis. A person may exhibit what is known as ARC or AIDS-related complex, serious in itself, or progress to full blown AIDS. The symptoms include extreme fatigue, night sweats, chills, fever, swollen lymph glands and spleen, loss of appetite and weight

loss, diarrhea, and depression. Most AIDS victims, if they live long enough, will develop cancers such as Kaposi's sarcoma (tumors of the blood vessel tissue in the skin and various organs). Later stages of AIDS also can produce dementia. AIDS is to date more than 90% fatal.

The HIV virus is spread through body fluids primarily blood and semen. At first the primary populations at risk were homosexual males and intravenous drug users. There was also a higher incidence of AIDS in Haitians, prostitutes, children born to mothers with AIDS, and persons who received blood transfusions prior to 1985. As the spread of AIDS in the homosexual population leveled off, it continues to grow in the heterosexual population. Emerging Central African nations such as Zaire have an extremely high segment of their populations infected with HIV 2. Most recently the AIDS epidemic has shown a menacing increase in the newly recognized nations originally part of the U.S.S.R.

AIDS was initially an orphan condition with very little public interest or monetary support for research. However, the deaths and infections of some prominent actors, politicians and artists together with a loud outcry from the homosexual community brought AIDS to the forefront of research and public attention. It should be mentioned that other research into retroviruses was burgeoning at the time due to the discovery of oncogenes possibly caused by retroviruses. All combined to focus the world's attention. Central African nations are experiencing an explosive HIV-2 epidemic. Public information campaigns, most notably the blanketing of the U.S. by the then Surgeon General C. Everett Koop, made everyone aware that the best prophylactic for AIDS was "safe sex", that is, condoms, monogomy and/or abstinence. Screening techniques for blood samples were developed. Tests for the HIV virus, though not 100% accurate, are now available. In addition, regulations for providing certain drugs sooner than usual (after Phase I clinical trials) were waived with medications which showed low toxicity and strong promise of efficacy. This has had many medical and politicial ramifications.

There are three fronts of chemotherapeutic attack: nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors.

NRTIs -
- ziduvidine (ZDV (formerly AZT), Retrovir®)
- didanosine (ddI, dideoxyinosine, Videx®)
- zalcitabine (ddC, dideoxycytidine, Hivid®)
- stavudine (d4T, Zerit®) and
- lamivudine (3TC, Epivir®)

NNRTIs -
- nevirapine (Viramune®)

Protease Inhibitors -
- saquinavir (Invirase®)
- ritonavir (Norvir®)
- indinavir (Crixivan®).

The NNRTIs have fewer side effects than the nucleoside analogues.

"Cocktails" of various drugs have been devised as well. Resistance to various drugs such as AZT has been observed.

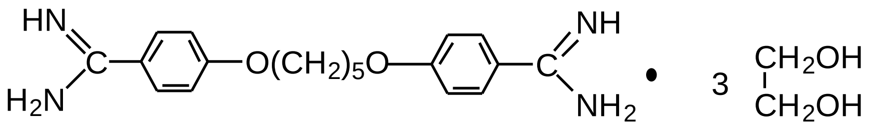
There has been research into possible vaccines for HIV which has been plagued with problems due to the rapid adaptation of the virus by changing its coat protein (the antigen). Within the next few years, at least 27 new vaccines will reach clinical trials. More promising is the use of T cell receptor protein CD_4 which can be produced through recombinant DNA techniques. The recombinant CD4 can act as a "Judas goat" binding to HIV before it can attack the target T-cells.

The politics of AIDS are also very interesting. Originally classified as an Orphan Disease, the drugs being developed for its treatment fell under the mantel of the Orphan Drug Act which would give exclusive marketing rights and extended patents to a single company. In the case of AZT Burroughs Wellcome was charging about $3000 per year for the 500mg per day prescription. This has brought outrage from many sectors which have sought to reclassify AIDS as a disease or to limit the patent term of AZT.

Stopping HIV is not the only avenue for research. Much work is proceeding on drugs to treat or prevent the opportunistic infections which prey upon immune deficient victims. Aerosol pentamidine was approved several years ago for the prophylaxis and treatment of ***pneumocystis*** infection.

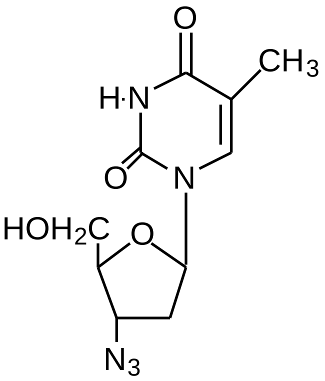
Corticosteroids and a combination of clindamycin (antibiotic) and primaquine (an antimalarial) have also been used to treat pneumonia.

We will carry AIDS infections with us into the 21st century. Currently the epidemic has grown and spread to various populations. According to current government figures, as many as 1.5 million Americans may be infected with HIV. 95,000 to 200,000 of these may already be experiencing the first stages of the disease. More time, research and money are needed to send this scourge the route of smallpox and polio. The course of chemotherapy is expensive. Developing countries need financial relief to stem the epidemic not only of AIDS but also of all the opportunistic infections which can result from weakened immune systems.

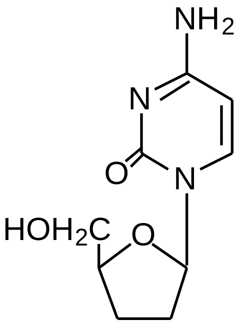


pentamidine (300 mg every 4 weeks)

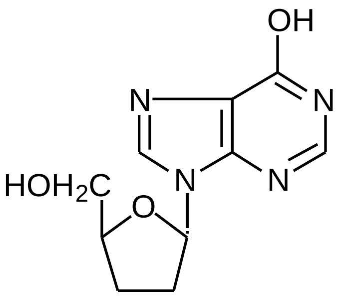
Side Effects: cough (38%), bronchospasm (15%), fatigue, metallic taste, shortness of breat, loss of appetite (53-72%), dizziness, rash (31-45%)



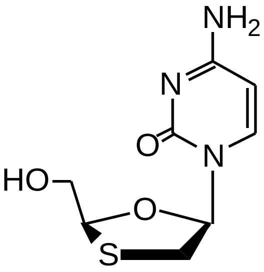
3'-α -azido-2',3'-dideoxythymidine
AZT



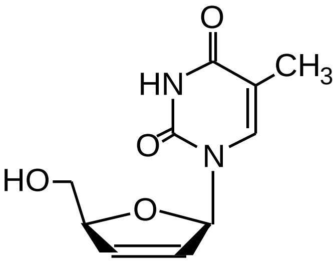
2',3'-dideoxycytidine
ddC



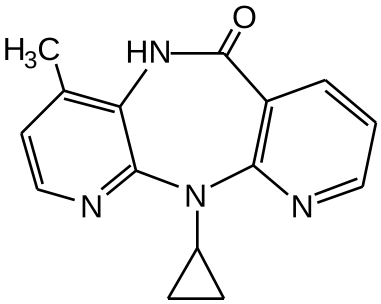
2',3'-dideoxyinosine
ddI



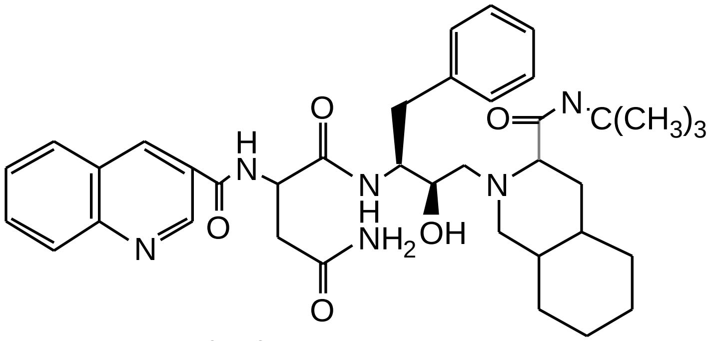
lamivudine
Epivir®



stavudine
Zerit®



nevirapine
Viramune®



saquinavir
Invirase®